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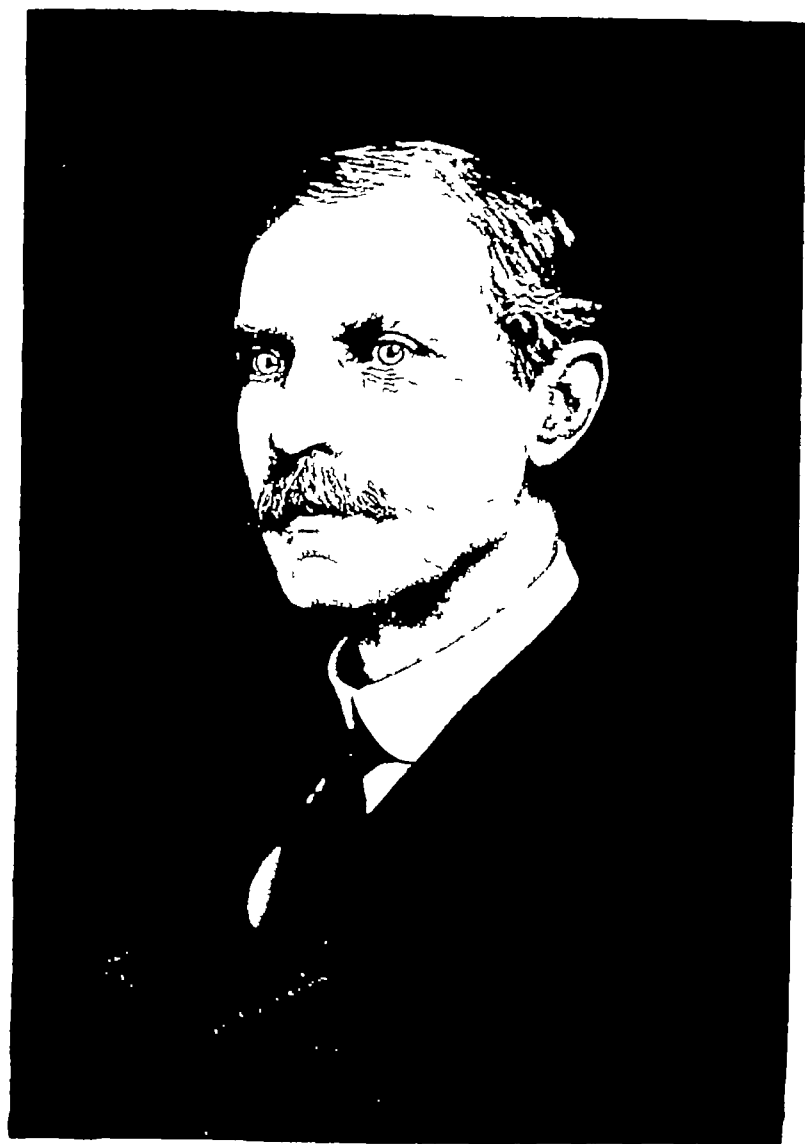
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J. N. Langley

JOHN NEWPORT LANGLEY IN MEMORIAM

ON October 31st, Professor Langley, after giving an early lecture to a large class of students and after examining the results of a long experiment done on the previous day, became unwell. Pneumonia followed, and he died at his home on November 5th. He had been controller and editor of this *Journal* since 1894, and it is fitting that an account of his life and work should appear in these pages.

JOHN NEWPORT LANGLEY was born at Newbury in 1852, the second son of John Langley, a private schoolmaster, and of Mary, eldest daughter of Richard Groom, assistant secretary to the Tax Office, Somerset House. He was educated at home and at the Exeter Grammar School, of which his uncle, the Rev H Newport, was Head Master. He entered St John's College, Cambridge, in October, 1871. During his first five terms he read mathematics, to which he added constitutional history and other literary subjects, with the intention of competing later for a place in the Civil Service, at home or in India. Among his chief friends of his own year in College were R. F. Scott, now Sir Robert Scott, Master of St John's, Milnes Marshall, who became Professor of Zoology in Owens College, Manchester, and C. T. Clough, who later joined the Geological Survey of Scotland.

During his second year he changed his plans and gave up other subjects to read Natural Science. This was due in part, no doubt, to the growing influence of these friends of his, but he was drawn that way chiefly because he then first came into contact with Dr Michael Foster, who as Fellow, and Praelector of Physiology, of Trinity College, was at that time conducting classes in Elementary Biology, Embryology and Physiology in a small room lent by the University and furnished by Trinity College. Langley first attended Foster's lectures and practical classes in May, 1873. Teacher and subject alike held him, and from that month until his last days, without any break or important pause and without leaving Cambridge, he gave all his working life to the service of physiology.

Foster himself, then 37 years of age, had only four years before been brought by Trinity College to Cambridge from University College, London. He became the first Professor of Physiology in the University

in 1883 and was well known in wider circles later as Sir Michael Foster, Secretary of the Royal Society, founder and first editor of this *Journal*, and a prime mover, too, in the foundation both of the Physiological Society and of the International Congress of Physiologists. The story of his life has been well told in this *Journal* by Langley himself in the number for March, 1907, vol xxxv. At the time when Langley first entered his class, Foster was already showing his powers of attracting and selecting the best recruits for biological science from among men of most diverse kinds and origins—powers which seem so astonishing when their far-reaching results are seen in retrospect, yet not so astonishing to those who can vividly recall the personal charm, the humour and the shrewdness of Foster himself, and the reflection of all these in his presentment of any subject.

More than twenty years later Foster wrote of his meeting Langley, "From the very first I marked him, as a man of whom something was to be made." Langley himself, forty years after those early days, wrote that "Foster led a considerable number of his early pupils to a scientific career. He first aroused an interest in scientific problems and then, sometimes gradually, sometimes suddenly, suggested that there was no better course in life than that of trying to solve them." So far as Langley was concerned it seems clear that the change from an arid mixture of lecture room mathematics and history to the living realities expounded by Foster brought not only new direction but new energy. In the following year he was elected to a Foundation Scholarship at St John's, and in December, 1874, he was placed in the First Class of the Natural Sciences Tripos, together with his friends Milnes Marshall and Clough of St John's, and Carpenter of Trinity, later Assistant Master at Eton. He had already begun to help Dr Newell Martin, then Foster's Assistant, in the practical classes and in 1875, having taken his B.A. degree, he became Demonstrator in succession to Martin, who had left Cambridge for Baltimore, to be Professor in Johns Hopkins University, carrying with him to the United States many fertile seeds to which the lineage there of Foster's ideals and methods may be clearly retraced. In 1877 Langley was elected to a Fellowship at Trinity College, which had been thrown open to competition by men of other colleges, and Trinity was thenceforward to be his home.

As Foster's Demonstrator, from 1875 until 1884, Langley was responsible for the organisation and conduct of all the laboratory class work, while the number of students in physiology was mounting from units to scores. Even before taking his degree he had begun, at

Foster's suggestion, a study of the action of jaborandi (pilocarpine) on the heart. The results were given in his first published paper, read before the Cambridge Philosophical Society in April 1875. This was reprinted in the *Studies from the Physiological Laboratory in the University of Cambridge* for 1876, of which three parts were published, in 1873, 1876 and 1877. Here his work appears side by side with papers by F. M. Balfour, Milnes Marshall, Newell Martin, Dew Smith, S. H. Vines and W. H. Gaskell. These were the chief members of that brilliant band of young workers, each one of them destined for work of enduring value, to which he was now joined. Those published studies, in most part collected from various Journals, appeared as edited by "The Trinity Praelector in Physiology," under grey covers, bearing the arms of that College. They have interest here because they proved to be the immediate forerunner of the *Journal of Physiology* which succeeded them in March, 1878. It is interesting now, too, to note that Foster, in introducing the first part of these *Studies* thanks the University "for having permitted me, a simple College Lecturer, to occupy, at some inconvenience I fear to others, the two University rooms in which my lectures are given, the practical teaching of my class conducted and the physiological work carried on. I have presumed on their kindness and ventured to call these rooms the Physiological Laboratory in the University of Cambridge." In these two small rooms, used both for teaching and for histological, chemical and experimental research work, began Langley's progressive occupations as student, as teacher and as investigator. He was destined to see those rooms overcrowded and extended, to pass to a new suite of rooms, to see those expanded again by large additions in 1890 into a well-equipped school, and to move finally from that into the present Drapers' School of Physiology in 1914. He was almost from the first Foster's chief lieutenant, he was to succeed him as Professor and as Editor of the *Journal*, he was to become a master investigator. As Foster had seen when meeting him as a youth of 20, he was indeed "a man of whom something was to be made."

Langley's career was in fact settled with unusual finality, as events were to show, in that May term of 1873. He then began a continuous line of work which was pursued without a gap for over fifty years up to the last days of life. From it he never looked aside at all, even in the direction of gaining a medical qualification, nor did he ever, as years advanced, allow administrative or public work to take the place of new inquiry, or to break the thread of his researches at any point. The external events of his life, therefore, are easy to tell: they follow and

mark the unbroken tale of his research work. They may be set down at once in order here. We have seen that in 1875 he took his degree and was already assisting Foster in his practical courses of teaching. In that year he collaborated with Foster in producing *A Course of Practical Elementary Physiology and Histology* (Macmillan & Co.). In 1877 he had been elected to a Fellowship at Trinity College. In 1884 he was appointed to the permanent College staff as a Lecturer in Natural Science, and in the same year he became University Lecturer in Histology. Those posts he held until 1903, when he was elected Professor of Physiology in the University upon Foster's resignation of the Chair, having served already as deputy Professor from 1900. From 1875 to 1900 he had lectured in each of three terms every year and held classes for advanced students in histology. From 1900 for exactly another quarter of a century he lectured to elementary students, first as Deputy Professor, then as Professor, at least three days a week in every term and gave demonstrations to them, in addition to courses of advanced teaching and in addition to his other work as head of a great School.

The main achievements of Langley's research work are familiar to readers of the *Journal of Physiology*, and need little here in the way either of description or comment. They stand permanently in their place not merely as additions here and there to knowledge, but as indispensable stepping stones along which, at this point or that, the progress of knowledge has actually made its way. Each gain he made was a step placed securely and finally, and few indeed of them, as the road has become more firmly and widely trodden by others following, have been found wrongly placed. All his chief works keep, and must always keep, their place in the significant history of animal physiology.

The full tale of his published work is given in order below. The bare titles of his papers and books, deployed there along the years, give the plainest testimony to his unwearying, unhalting, service to science. No single year in all that series, extending well nigh for half a century from youth to age, appears without its contribution of effective work.

There is a strong call made both to heart and mind in such a review as this of the achieved work, when seen as a whole, of the life of any man of science. Interest and instruction are found in noting the apparent accidents which lead inquiry hither and thither, or in tracing a continuity either of idea or method through a long sequence of researches in widely varied objective fields. However it may be for other branches of science, it may be said with much truth that in the history of physiology the greater the pioneer the clearer is the line of the continuous, if devious,

clue he may be seen to have followed. In the work of Langley, what in retrospect is seen as obvious continuity runs through all his main inquiries from the beginning to the distant end. He worked from point to point, as knowledge came from his own labours. He was not among those who pass restlessly from one part of the field to another to join searchers elsewhere who are finding success.

In broad outline his contributions to physiology may be considered as in two main groups, those dealing with the mechanism of secretion and those establishing the main anatomical and functional lines of the autonomic nervous system. The former belong to his first fifteen years of work, the latter to the succeeding thirty and more. Chance, as it seems, gave the starting point. Foster in 1874 suggested his examining the newly introduced drug *jaborandi* (pilocarpine) and its effects on the heart. This he did, for frogs and for mammals. This led directly to study of its effects upon secretion, first in the sub-maxillary gland of the dog, and to the researches into the physiology of secretion which followed in close succession until 1890. It was in this earlier work that he exhibited and developed his salient qualities. A merely competent worker might have described the action of pilocarpine and then have passed, even usefully, to the actions of similar and different drugs. Langley, however, opened up at every point the physiology of secretion, adapting his technical work not to convenience but to his intellectual needs. He made histological studies of gland structure, in activity and rest, checking the interpretation of the appearances of killed and stained cells by simple, but most effective, direct observation of the living gland cells. These were correlated with the experimental unravelling of the nervous influences upon glands, and the share in these taken by the vaso-motor phenomena. Both sets of results were linked again by chemical estimations of the changing qualities of the secretion. The well-known steps he took and the variety of work done are to be traced in the titles of his successive papers. Before his work the accepted view had been that of Heidenhain, that gland cells became more granular as secretion took place. Langley showed that the reverse was true, that granules were stored up during rest, to be passed out in secretion, not only in the pancreas, as Kühne and Lea¹ had shown, but over a wide range of glands. By his study of the secretory nerves Langley moreover showed that the belief in the existence of special "trophic" or secretory fibres rested on evidence which could be explained in terms of purely vaso-motor changes in the

¹ Shendan Lea, of Trinity College, later Fellow of Caius, an immediate contemporary of Langley.

gland circulation His results, whether finally decisive or not, still hold the field so far as that argument is concerned, and must in any case always have permanent value of their own The chief results of his work upon secretory glands were gathered up and incorporated in the valuable article he contributed upon the "Salivary Glands" to Schäfer's *Text-Book of Physiology*, 1898

Midway in these studies of secretion Langley had turned aside to make a histological report upon the brain of a dog in which Goltz had removed part of the cerebral cortex and which had been shown at the International Medical Congress of 1881 This led him on to work with Sherrington upon the degeneration of nerve-tracts after removal of the cortex, and later to a study (with Grunbaum) of the degenerations in another dog upon which Goltz had operated

By 1890 Langley had passed on to the next and most conspicuous phases of his work It was natural that the experimental studies of sympathetic and cranial nerve fibres to the salivary glands should have led him to use the same technical methods in the exploration of other functions of those systems of nerve fibres To this two other factors contributed In the years from 1886 to 1889 Gaskell, five years senior to Langley, had already made his great simplification of bringing into one system, for which he introduced the word "visceral," not only the nerve fibres of the sympathetic system, which, as he showed, spring from the thoracic and upper lumbar regions, but also those from cranial or from sacral nerve roots All these alike, at least in their efferent fibres, are small medullated fibres and may be considered as one system, interrupted at two points along the axis of the central nervous system by the outflows of fibres, "somatic" as opposed to "visceral," for the arm and for the leg The cranial and sacral fibres were apparently alike in being antagonistic in function to the sympathetic fibres Gaskell's generalisations, fundamental and illuminating as they were, left very many essential points of anatomical and physiological importance unsettled, and many of these were just of the kind which Langley was already confronting in his own work and for which both his technical equipment and his temperament alike exactly fitted him The second factor was the accidental opportunity of trying the effects of another new drug Just as pilocarpine had led to his studies of secretion, so now a supply of pitiuri (nicotine) put into his hands by Professor Liversidge, gave him, as he soon found, a novel and potent instrument, used as he used it, for unravelling the structure and functions of the sympathetic and of the other two parts of the "visceral" or "autonomic" system

So began that long series of well-known studies, seen in perspective as the eye scans in a list the titles of papers that pass in opulent procession through the years. They deal intensively with the details of the autonomic system for some fifteen years after 1890, and thereafter pick up and follow particular lines of inquiry branching at some point from the main stem.

There has been an occasional tendency in some writers of recent years, not indeed to belittle the value of Langley's additions to knowledge in this field, for at almost every point those stand unshaken and secure, but to represent them as counting for not more than the completion of the details of a scheme of which Gaskell had conceived the framework and had laid down all the fundamental principles. Gaskell's important advances in this field were made as already mentioned between 1886 and 1889. Thereafter he passed to his well-known studies of the origin of vertebrate animals. It is true that by his work on the visceral nervous system, just as by his investigations of the heart and of the heart-beat, Gaskell had given a new orientation to ideas and a new basis for all future work. But no more in the one instance than in the other is it right to suggest that the structure of later knowledge, built up in the case of the visceral nervous system so largely by Langley's masterly skill and tireless industry, lay implicit in the foundations so firmly traced by Gaskell. To some extent the suggestion is based perhaps on a misreading of Gaskell's own book on the *Involuntary Nervous System*, published in 1916. In this he gives a summary of the knowledge available at that date, following an account of his own early work up to 1889, the treatment was not intended to be historical and gave no occasion for drawing distinctions between views justified in 1889 and those taken later. As Langley himself wrote, in the cordial and well-informed tribute which he paid to Gaskell's work on his death (*Proc Roy Soc*, 1915, 88 xxxi) "Gaskell's work clarified the air. It gave anatomists and physiologists a clearer view of the general arrangement of the efferent nerves governing unstriated muscle and glands, and it directed the attention of physiologists to points which they had singularly neglected." When Langley turned his own work in this direction in 1890, our knowledge of the sympathetic system, in spite of Gaskell's inspiring contributions, was still in much dark confusion. It was believed that its efferent fibres might have one or many nerve cells, or none, on their course. By experimental work with nicotine, by degeneration experiments and by histological observation, Langley acquired such multiplied data that we may now believe there is one

nerve cell and one alone, between the central nervous system and the periphery for each nerve fibre. The posterior root ganglia were held to contain visceral efferent as well as sensory nerve cells, that was shown not to be so, and sensory and motor nerve cells were shown not to occur in the same ganglia. It was believed by many, especially by French workers, that true reflex actions could be obtained from peripheral ganglia, Langley showed that the facts could be explained by his "axon-reflexes." By investigating the pilomotor fibres he worked out the segmental distribution of the sympathetic fibres to the skin and their relation to the sensory fibres of the corresponding spinal nerves. There was no general plan of the distribution of nerve fibres of the white rami to the successive ganglia, and this Langley effectively made. Gaskell's fruitful conception of the visceral or autonomic system as a single system of uniform origin, interrupted only by the limb centres, has been modified essentially. To the newer conception of the cranial and sacral outflows of visceral nerve-fibres and their connected ganglia, as belonging to a system, which Langley called "oro-anal," different in origin, distribution and function from the thoracico-lumbar sympathetic system, Langley's physiological analysis contributed in harmony with Gaskell's later and chiefly morphological work. These studies of the autonomic system by Langley, together with the earlier studies of Gaskell, to which they provided exactly the complement required, must always remain among the greatest of the achievements of English physiology.

Important as were the broad generalisations thus established or enriched by Langley's work, tribute must be paid to the great beauty and interest of very many of the individual researches within the series. His studies with H. K. Anderson, now Master of Caius, of the nervous and muscular mechanisms of the iris, his demonstrations of the pilomotor mechanisms and their equivalents for the feathers of birds, his unravelling, again with Anderson, of the intricate nervous supplies to the various pelvic organs, his proof of the general functional similarity between all efferent nerve-fibres by successful cross-unions of bulbar-autonomic, of sympathetic and of somatic nerves, after which each was shown to take on after regeneration the functions of another, of these or of many other researches each physiologist will make his own choice for special admiration.

From 1905 onwards Langley turned close attention to the mode of functional union between nerve-fibre and muscle substance. Here he gave another extension to his analytic use of drugs in experiments. Curari and similar drugs had been generally held to exert their actions

upon the last terminal branches of the nerve-fibre and to be without action on the tissue in which the fibre ended. Langley had shown that nicotine stimulates peripheral nerve-cells after degeneration of the pre-ganglionic fibres ending in them. In the peripheral tissues the persistence of the action of adrenaline, which had been demonstrated by Levandowsky, and by himself, after degeneration of the sympathetic fibres, made its point of action appear to be a hypothetical neuro-muscular "junction," as Elliott had concluded already from his work at the same time in the Cambridge laboratory. Langley found that nicotine applied directly to certain muscles gave a contraction limited to the area just under or near the nerve-ending, and that this localised contraction was prevented by curari. These and other observations led him to suppose that the region just under a nerve-ending which he called the "neural region," is specially excitable, probably because 'receptive substances' are there, or are liberated there. He concluded that the specific action of most poisons depends on the nature of the receptive substance present, while some poisons may have in addition their own action upon the actual end branches of the nerve-fibre itself. His results in this field of work are given in his Croonian Lecture before the Royal Society in 1906, and received further development in the years following.

In 1910 the Drapers' Company of London offered to build a new School of Physiology in the University. By that time the old physiological laboratory which Foster had greatly enlarged in 1890 was quite inadequate for the increasing numbers of students, and almost ludicrously inadequate for the needs of the research work being done in many different parts of physiology and biochemistry by various members of Langley's staff and their juniors. Langley gave close attention to the design and equipment of the new School as it was being planned by the architect Sir Thomas Jackson, and his own research work at that time, though active, was confined chiefly to points of detail. He worked out, however, an ingenious method of blocking given areas of the peripheral blood circulation by the injection of starch granules of selected size, and of showing the exact anatomical basis of the experimental results by subsequent staining of the granules *in situ*. The new School was formally opened by Prince Arthur of Connaught in June, 1914. It had been planned so as to share a large new lecture theatre with a future building for Biochemistry on part of the same site, funds for this were not then available, and lectures to the large elementary classes continued to be given at some distance away in the large theatre of Foster's building. Biochemistry in 1920 received a benefaction on a scale undreamed of in

1914 and was established on a larger plan and a different site. The distance between the old lecture theatre and the new Drapers' School added much to the fatigue of the elementary teaching which Langley maintained up to the last.

The provision of this great building was a fitting recognition of the distinction and activity of the school of workers which Langley was leading, for the first time they were to have reasonable space and equipment for investigation, and it was with high hopes that Langley passed over with them to its occupation. Within a few weeks, however, the War began. In coming it brought relative emptiness to the building, it was to bring also irreparable losses to that group of workers, but it was to prove to be only a postponement of hope and achievement.

During the War, Langley turned his own studies into directions likely to have medical value. He investigated in particular the changes in muscle after section of the motor nerves, the modes in which atrophy followed and the effects upon it of massage and electrical stimulation. All his junior colleagues were upon other forms of war service, but he was assisted in these, and in other studies of nervous degeneration and of the anatomy of nerve trunks, by various Japanese workers. In the succeeding years, as the tide of students returned after the War and colleagues were regained, he turned to gathering up and supplementing his main body of work upon the autonomic system, and he prepared his book *The Autonomic Nervous System* of which Part I was published in 1921. This was soon translated into both French and German. In the last years he studied again the secretory activity of skin glands, and published a series of papers upon the capillary circulation and its nervous control in vascular reflexes. On the day before his last short illness he was engaged in a long experiment which kept him busily and continuously at work for six hours. In this he seemed to his laboratory assistant, who had helped him for nearly forty years, to be as strenuous in concentrated observation and as skilful in his handiwork as at any time during that long tale of work.

Langley's investigations gained early recognition and many appropriate honours came to him. In 1883 he was elected into the Royal Society, at the age of 31. In 1892 he received a Royal Medal from the Society. He served upon the Council for two periods and was Vice-President in 1904-5. In 1893 he was President of the Neurological Society of Great Britain. He received the Baly Medal of the Royal College of Physicians in 1903, and in 1912 the Retzius Medal of the Swedish Society of Physicians. Honorary degrees were conferred on him

at Dublin, St Andrew's, Groningen and Strasbourg, and he was honorary member of almost every important academy of physiology or medicine in other countries

In person he was of middle stature, always spare and upright in figure, trim and well-balanced in carriage. He dressed well, and therefore inconspicuously, he followed always the newest fashion of dress, but after middle-age at the appropriate distance. His photograph shows well the strong lines of his face, and would serve for almost any period in the memory of his oldest living pupils, because age seemed to bring no more change to his face than to his figure and step, save for a late greying of his hair. Yet no photograph could show his most characteristic and impressive feature, the steely blue-grey colour of his eyes which gave them a singularly arresting quality.

He had no affectations either in the lecture theatre or the laboratory, he kept strictly to the business in hand but showed, when fitting, the same friendliness and humour as in his ordinary social intercourse. To his pupils, old or young, as to his closest friends, he offered a kindly interest often warmed with humour, yet without allowing any intimate touch upon a sensitive inner reserve which he instinctively maintained. Save for that ultimate reserve, he stood in no way upon his dignity as professor, any more than in private life, he cared for the position but not for the title of professor, and no man indulged less in the solemnity of office. In informal demonstrations he was at his best as a teacher, and would show endless patience in explaining difficulties to any keen student, however stupid. In formal lecturing he was not so successful, in spite of his immense experience, he had not that touch of dramatic art, and he was too critical to have that selective dogmatism, which go together to make an effective elementary lecturer. His chief and lasting influence upon his pupils was by the example of his work and by direct precept in the technical directions in which he was a master craftsman.

To many of his pupils, and indeed to very many others outside that circle, he gave help of another kind, he taught them how a scientific paper should be written. Here we come to that part of his life's work by which he gave a less direct but inestimable service to physiology both in this country and in others. Beyond all his work as a teacher and leader in Cambridge and beyond his maintained labours as investigator, Langley undertook the heavy and unrelenting task of editing the *Journal of Physiology* for more than thirty years, and he so interpreted and performed his duty in that respect as to bring aid, seen or unseen, to other workers all over the world.

The *Journal* was founded by Foster in 1878, as we have seen. In 1894 it was becoming increasingly in debt and was threatened with extinction. Langley in that year arranged to pay off the debt, a considerable sum, and to receive the unsold stock. Thenceforward and until his death he owned and edited the *Journal*, though Foster's name was retained upon the cover during his lifetime. From the beginning Langley aimed at two ideals. The first was to admit nothing that was either not new or not making some definite advance in knowledge upon the strength of valid evidence adequately presented. The second was to economise space and to save the time of workers everywhere by insisting that each paper admitted should be pruned of every redundancy not needed to satisfy that first condition. To gain these two ends in the general interest, he spared neither time nor pains. The remuneration he earned as owner of the *Journal* was small recompense for his heavy labours as editor. In the midst of the work of teaching and research already described, work which would have more than filled a lifetime for most men, he bore continuously this extra burden of reading, of editorial correspondence and of press management. His methods were never arbitrary, his judgment of papers was most conscientious and deliberate, based on wide knowledge of work already published, supplemented at need by that of his colleagues. Unlike most editors, however, for him the acceptance of a paper meant often not the end but the beginning of a task. He thought it due to the *Journal* and to science that the paper should be cast in the most effective form and reduced, compatibly with that, to the least size. This meant correction, often heavy correction, commonly suggested in detail by himself, and it involved the exchange of views with sensitive and sometimes irascible authors. Not a few workers have been surprised to receive their paper again in manuscript almost entirely rewritten by Langley. Those to whom this may have come as an unwelcome shock were those who afterwards had most cause of gratitude to find for the service done them. On this side of the work he often showed signal generosity. For younger men, and especially for foreign contributors, he would often completely recast a paper, amend or extend references, revise tables, draft conclusions, and would accept no published acknowledgment. With some authors he had difficulties, of course, though it is doubtful whether in any instance his judgment of their failings in this sphere or his proposed remedy for them was shown to be faulty. At least it may be said that if an occasional contributor was vexed, the immense majority were well served and grateful, while Langley put the whole body of workers using the *Journal* into his lasting debt. He made,

and he now leaves, the *Journal of Physiology* unsurpassed in the high standards maintained both as to the content and as to the form of the papers within it. He was fortified in his difficult duties by very strong and impersonal feeling. This could not possibly be better expressed than in words he used himself in the course of his address as President of the Physiological Section, at the Dover meeting of the British Association in 1899. There he said, "Those who have occasion to enter into the depths of what is oddly, if generously, called the literature of a scientific subject, alone know the difficulty of emerging with an unsoured disposition. The multitudinous facts presented by each corner of Nature form in large part the scientific man's burden to-day, and restrict him more and more, willy-nilly, to a narrower and narrower specialism. But that is not the whole of his burden. Much that he is forced to read consists of records of defective experiments, confused statements of results, wearisome description of detail, and unnecessarily protracted discussion of unnecessary hypotheses. The publication of such matter is a serious injury to the man of science, it absorbs the scanty funds of his libraries, and steals away his poor hours of leisure." The man of science in his defence against this kind of injury has never had a more determined or untiring champion than the speaker of those words.

In spite of his continuous industry in the laboratory and the special calls upon his time made by the *Journal*, Langley found time to touch life at many other points. In his early graduate days he belonged to the Chit Chat Society, now extinct, of which each member in turn read a paper for discussion in his own rooms, and here he met men like the Lytteltons, the Balfours, J. K. Stephen, M. R. James, A. C. Benson and others, on fields far from physiology. For more than thirty years he was a member of the "Family," a dining club of twelve Cambridge residents, of ancient and probably Jacobite origin, which meets on alternate Fridays in full term. He belonged also to the "Ambarum," another dining club, meeting (until the war) alternately at Oxford and Cambridge, and of this the members most nearly his contemporaries were Hallam Tennyson, Gerald Balfour, Lord Milner, J. G. Butcher (now Lord Danesfort), and Edmund Gosse. From the early eighties until his marriage in 1902 he lived in a large set of rooms in Whewell's Court, succeeding Dr. Lightfoot, Bishop of Durham, and here his older pupils have perhaps their most vivid memories of him. He enjoyed giving hospitality, especially in small luncheon or dinner parties, he was a good judge of the qualities of men, manners and talk, and no less a judge of the right uses of food and of wine. Genial even to gaiety on those occasions, he

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- 1873 LANGLEY On the Physiological Action of Jaborandi *Proc Camb Philos Soc*, April, 1875, p 402
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THE EFFECT OF STIMULATION OF THE VAGI ON THE PYLORIC REGION OF THE STOMACH

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THE course taken by the vagus fibres which supply the pyloric region has not been very definitely ascertained. Anatomically the main branches of the anterior and posterior vagal trunks from the œsophageal plexus are traceable to the region of the incisura angularis and their terminal fibres supply the pyloric antrum. In no instance does the anterior or posterior vagal trunk send fibres as far as the pylorus canal or sphincter. Many observers state, however, that all the pyloric region receives its nerve supply from these branches. The anterior vagal trunk has been shown to give off a branch, and sometimes two or three branches, to the liver, which communicate with sympathetic fibres and have been called the hepatic branch of the vagus. From these, nerve strands descend to supply the pylorus and first part of the duodenum. Latarjet(1) states that the pylorus receives its entire nerve supply, in man and the dog, from above and considers these strands to consist wholly of sympathetic fibres. One of us, McCrea(2), has dissected the hepatic branch in man, dog, cat and rabbit and shown that the strands given off to the pylorus contain vagus fibres. It seemed then desirable to ascertain the effects which this pyloric branch is capable of producing upon the musculature of the pyloric region.

In a recent paper, McCrea, McSwiney and Stopford(3), working on the intact stomach, have shown that stimulation of the vagi influences both the motor activity and "postural tonus," the type of reaction depending on the condition of the peripheral mechanism. The entogastric pressure was used as an indication of the state of activity of the peripheral mechanism, as this was found to vary with the degree of contraction of the viscus on its contents. In the passive organ (obtained by starving an animal for at least 12 hours) which did not normally maintain an entogastric pressure, stimulation of the nerve caused an augmentor response. In the active stomach (obtained by feeding a starved animal

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three hours before the experiment) a preliminary inhibitory response followed stimulation

Method The experiments were made on cats and dogs. The animals were anaesthetised with ether. The abdomen was opened by a mid-line incision extending downwards from the xyphoid process. The abdominal walls were retracted and the abdomen filled with warm saline. The pyloric region was severed from the rest of the stomach between two purse string sutures, just distal to the incisura angularis. A short vulcanite tube, in some instances with a balloon attached, was inserted into the pyloric antrum. The duodenum was ligated and care was taken to ascertain that all the nerves of the anterior vagal trunk running in the region of the lesser curvature had been severed. In some experiments a record of the movements of the body of the stomach was also taken by means of a tube passed into the stomach through the cervical oesophagus. The tube in the pyloric antrum was connected with a system filled with warm saline. A water float was used to record contractions and pressure variations. The vagi nerves were cut in the neck and stimulated with a faradic current of different strengths.

Investigations were also carried out on the movements as seen by X-ray, and photographs were taken when required. The animals having been anaesthetised the stomach was divided into two parts and the vagi prepared as described above. By means of a syringe, the bismuth sulphate was introduced into the pyloric region.

For observation on the inactive body of the stomach, food was withheld from the animals for a period of not less than 12 hours before examination, those animals, however, in which an active body of the stomach was required, received a small meal of meat extract and milk three hours before the experiment. We have not been able to control the activity of the pyloric region in the same manner as the body. The operative technique described effects the complete separation of the pars pylorica from the body of the stomach, while the nerve supply to the pyloric canal through the hepatic branch of the vagus remains intact.

Stimulation of the peripheral end of the vagus in the neck

The effect of stimulation varied according to the condition of the pyloric antrum. If inactive, the pyloric region was quiescent in the cat and slow spontaneous contractions were present in the dog. In the active condition, rapid rhythmic movements were observed in the pylorus of the cat and dog.

Stimulation of the nerve when the region was inactive initiated

rhythmic movements in the pars pylorica of the cat and increased the rate of contractions in the dog (Fig 2) On the other hand, in the active pylorus, rhythmic movements were always inhibited (Fig 4) The movements were decreased in rate but the height of the contractions was not affected The diastolic intra-pyloric pressure remained constant

In the cat stimulation of the vagus with moderate strength of current (just felt on the tongue) initiated peristaltic waves in the pyloric region which spread to the duodenum The frequency of these movements was approximately nine to ten a minute The contractions were of equal height and returned to the base, *i e* there was no variation in tone On increasing the strength of the current the character of the movements altered (Fig 1)

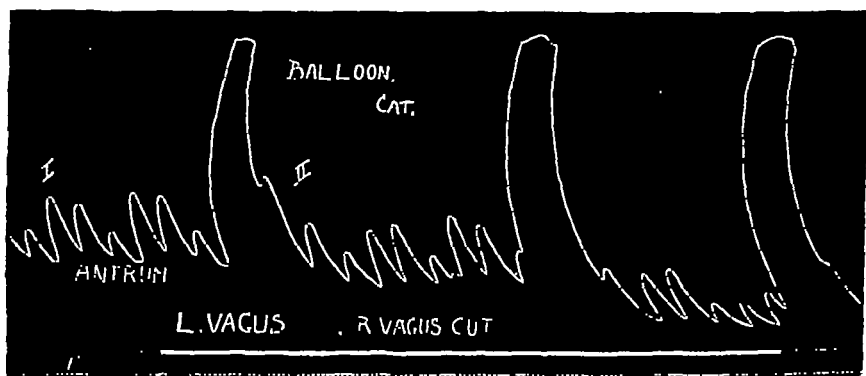


Fig 1 (1) Rhythmic peristaltic movements, and (2) initiation of total contractions on stimulation of peripheral end of left vagus

Large rhythmic contraction now occurred at intervals of 20 to 30 seconds On direct observation they were seen to represent a simultaneous contraction of the whole pyloric region In the intervals of these contractions, shallow peristaltic waves were seen, passing along the pyloric canal, the average frequency being about eight to twelve a minute This two-phase type of contraction, if established, persists throughout the experiment, and we have failed to re-establish the original one-phase type Similar movements have been described by McCrea, McSwiney, Morison and Stopford(4) in their X-ray investigations of the normal movement of the cat's stomach, and by Alvarez(5) in his investigations of the rabbit and cat

In the dog, the total contraction of the pyloric region was most frequently obtained, the whole pyloric region, rhythmically contracting

and relaxing at intervals of 20 to 30 seconds. The movements are remarkably regular, the amplitude, duration and frequency remaining

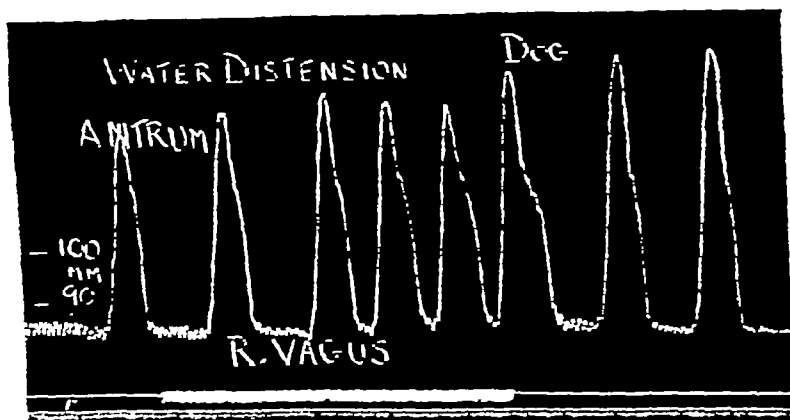


Fig. 2 Inactive pyloric region. Acceleration of rhythmic movements on stimulation of peripheral end of vagus nerve

unaltered as long as the pressure is kept constant. A strong stimulus may cause a sustained contraction, the rhythmic movements increasing in rate and starting above the base line, indicating summation. Escape may occur; the rhythmic movements become slower and the lever returns to the base line (Fig. 3). Occasionally shallow peristaltic waves, similar to those observed in the cat, occurred in the intervals of the total contractions.

Distension of the pyloric region of the cat ordinarily fails to initiate movements, whereas in the dog increase of content is usually an adequate stimulus. We have found an optimum tension exists, but if the walls of the pyloric region are over-distended by allowing excess of fluid to flow into the viscus, the amplitude of the contractions become diminished. These results agree with those of Ducceschi⁽⁶⁾ on the stomach of the dog *in situ*.

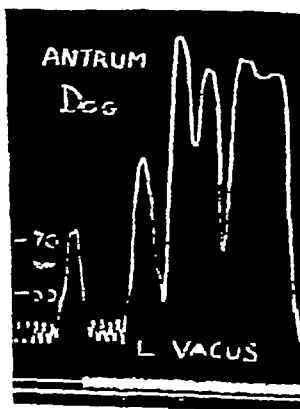


Fig. 3 Rise of lever from base line with rhythmic movements superimposed (spasm) on stimulation of peripheral end of left vagus nerve

In some experiments simultaneous tracings were taken from the body and pyloric region isolated from one another. The inactive body of the

starved animal shows a motor response on stimulation of the nerve, rhythmic movements were initiated and a rise of pressure occurred,

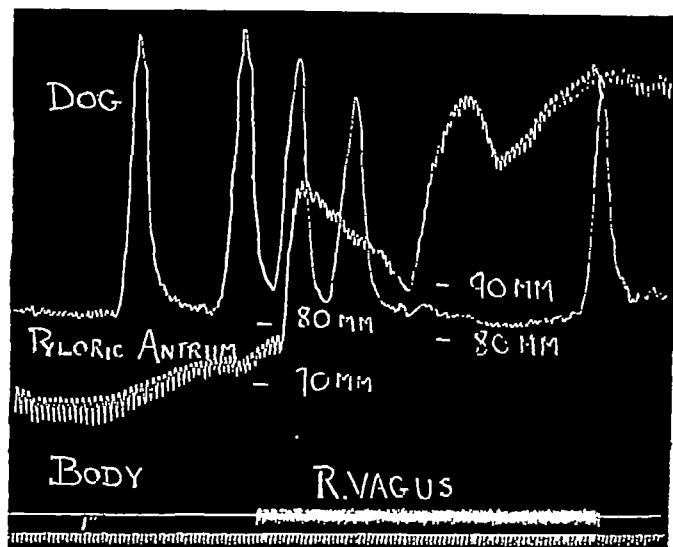


Fig 4. (1) Rise of pressure in body, and (2) inhibition of movements of pyloric antrum on stimulation of peripheral end of right vagus nerve

movements, if present, were augmented and sometimes accelerated (Fig 4). In the fed animal, vagal stimulation of the active body caused a fall of pressure and cessation of movements (Fig 5). In the pyloric region the effects were similar to those previously described.

In these experiments we have been able to show a complete dissociation in the response of the two regions. In some instances vagus stimulation caused inhibition of the cardiac end of the stomach and increased the rate of contractions in the pyloric region. We have also obtained the reverse effect, viz a motor effect in the body and inhibition of the pyloric region. The experiments of McCrea, McSwiney and Stopford on the intact stomach agree with these results.

X-ray observations The movements observed under X-ray and the response to vagal stimulation were in both the cat and dog the same as those previously described.

The results described above show that the nerve strands which descend from the hepatic branch of the vagus and run in the lesser omentum to the pylorus contain fibres for the pyloric canal and sphincter. These fibres control the augmentor and inhibitory response of this region, and

the nature of the response to vagal stimulation depends on the condition of activity of the pars pylorica. The recent work of McCrea, McSwiney

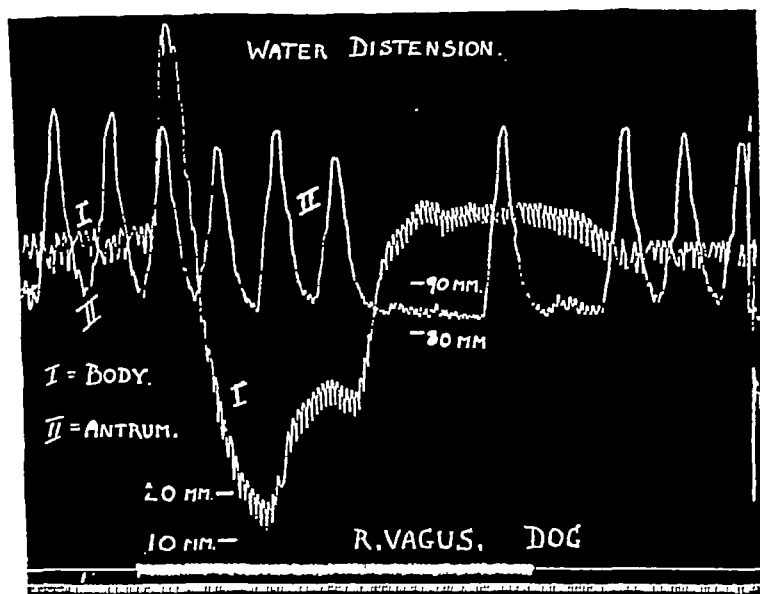


Fig 5 (1) Fall of pressure in body, and (2) inhibition of movements of pyloric antrum on stimulation of peripheral end of right vagus nerve

and Stopford affords evidence that the augmentor or inhibitor response of the stomach to nerve stimulation is controlled by the condition of the peripheral mechanism

It has been shown by a number of observers that in digestion the movements of the greater part of the cardiac end of the stomach differ essentially from those of the pyloric region. In the cardiac end, the contractions may be either rhythmic or tonic or both, while in the pyloric region the contractions are rhythmic only—peristaltic or total. The results we have obtained give additional evidence of this essential difference and it is particularly to be noted that in the pyloric region, stimulation of the vagi, whether it causes augmentation or inhibition of rhythmic movements, causes little or no variation in tone

CONCLUSIONS

- 1 The hepatic branch of the vagus supplies and regulates the pyloric region

starved animal shows a motor response on stimulation of the nerve, rhythmic movements were initiated and a rise of pressure occurred,

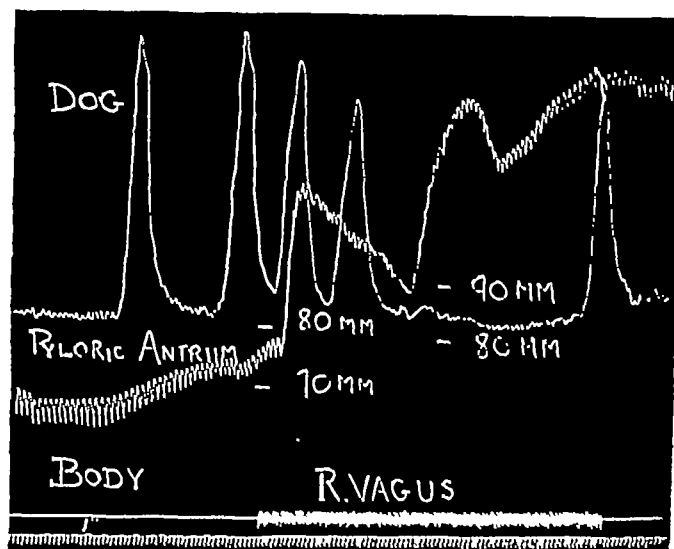


Fig 4 (1) Rise of pressure in body, and (2) inhibition of movements of pyloric antrum on stimulation of peripheral end of right vagus nerve

movements, if present, were augmented and sometimes accelerated (Fig 4) In the fed animal, vagal stimulation of the active body caused a fall of pressure and cessation of movements (Fig 5) In the pyloric region the effects were similar to those previously described

In these experiments we have been able to show a complete dissociation in the response of the two regions In some instances vagus stimulation caused inhibition of the cardiac end of the stomach and increased the rate of contractions in the pyloric region We have also obtained the reverse effect, viz a motor effect in the body and inhibition of the pyloric region The experiments of McCrea, McSwiney and Stopford on the intact stomach agree with these results

X-ray observations The movements observed under X-ray and the response to vagal stimulation were in both the cat and dog the same as those previously described

The results described above show that the nerve strands which descend from the hepatic branch of the vagus and run in the lesser omentum to the pylorus contain fibres for the pyloric canal and sphincter These fibres control the augmentor and inhibitory response of this region, and

2 Movements of the isolated pyloric region are similar to those observed in the pars pylorica of the intact stomach

3 The pyloric region does not exhibit the property of "postural adaptation"

The expenses of this investigation have been borne by a grant from the Government Grants Committee of the Royal Society

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subjects had been taking regular vigorous exercise for some weeks before the experiment and they may be called "untrained." The results of

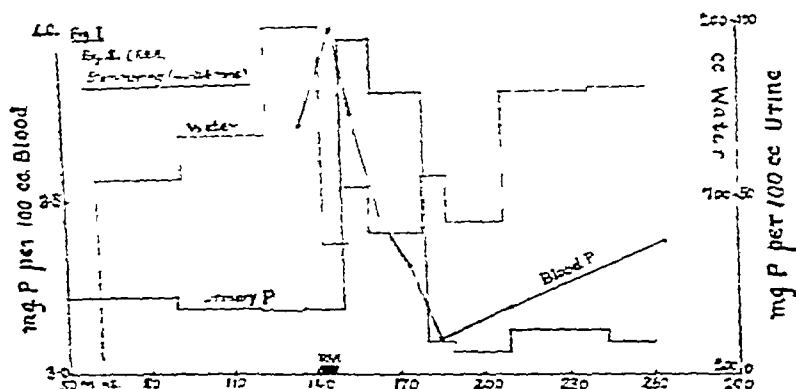


Fig 1.

these experiments are summarised in Table I. The "short exercise" was the vigorous stair running already described. A sample of blood was taken from one to five minutes after the cessation of exercise and the increase of the inorganic phosphate content of this sample above the "normal" value is expressed as a percentage in column "Rise per cent."

Exp 8. "Stair running" R.E.H.

Inorganic phosphate in blood. Mg. P per 100 c.c.

Time	a	b	Mean
135 mins.	3.72	3.72	3.72
142 "	Stair running for four minutes		
147 "	4.10	3.94	4.02
155 "	3.74	3.75	3.75
165 "	3.46	3.42	3.44
175 "	3.36	3.23	3.30
185 "	3.08	—	3.08
210 "	3.23	3.10	3.19
237 "	3.37	3.39	3.38

Inorganic phosphate in urine

Time of collection	Vol. c.c.	Mg. P per 100 c.c.	Rate P per hour mg	Rate H ₂ O per hour c.c.
55 mins	175	10	21.0	210
90 "	270	2.73	21.1	772
120 "	250	2.18	19.6	900
135 "	223	1.62	19.3	1172
150 "	128	3.22	19.3	592
160 "	122	13.23	95.0	732
180 "	204	13.45	82.3	612
190 "	131	1.24	9.7	786
200 "	312	1.01	6.7	690
242 "	564	1.02	10.5	1025
252 "	345	72	7.7	1035

Blood was drawn into a crucible containing 1.2 mg of sodium oxalate. A little over 1 c.c. was collected and two estimations were done, using 0.5 c.c. of blood for each. This slight modification of Briggs' method worked very well and enabled us, when necessary, to take a large number of samples at short intervals. Over 200 estimations were done in duplicate with an average error for a single observation of about 1 p.c. from the mean. In the later experiments samples of urine were taken at frequent intervals during the experiments and analysed for inorganic phosphate, ammonia, chlorides, and in some cases for other constituents. The phosphate was estimated by Briggs' method(5). The ammonia by a vacuum distillation method described by Stanford(6). The chlorides by Volhard's method. Urea was estimated by Marshall's method(7) and sulphates gravimetrically. A copious diuresis—500–1500 c.c. per hour—was produced by drinking large quantities of water. In this way it was possible to obtain samples at such short intervals as ten minutes, and by analysis to follow the rate of excretion of various constituents. It was demonstrated by Wigglesworth and Woodrow(8), and is our experience also, that this rate in the case of inorganic phosphate is quite independent of the extent of the water rate, provided the latter is above a certain minimal value (about 200 c.c. per hour). We found the same to be true of ammonia, but only approximately true for the other constituents investigated. It will be seen, however, that the variations in the rate of excretion due to the water rate were always insignificant compared to the variations under investigation. The behaviour of phosphates during water diuresis will be dealt with more fully in a subsequent paper.

Inorganic phosphate of blood

The typical behaviour of the blood phosphate curve in these experiments may be seen in Fig. 1. A small rise took place during and immediately following the exercise reaching a maximum, probably 3–4 minutes after the running had ceased. This was followed immediately by a rapid fall well below normal which attained its maximum value in about three quarters of an hour. A slow recovery to normal followed taking two to three hours for completion.

The typical behaviour of the rate of phosphate excretion may also be seen in Fig. 1. A sudden great increase, *after* the period of exercise is followed by a certain degree of suppression.

We publish in full the protocol of this experiment No. 8 selected from a group of fifteen experiments on ten different subjects. None of the

normal value and the lowest value obtained in any experiment. There is no guarantee that samples of blood were obtained exactly at the maxima and minima of the phosphate concentration curve. The later sampling was planned, however, from knowledge of the general form of the phosphate curve derived from earlier experiments, and it is unlikely that any large errors have been introduced.

The figures for all the urine determinations are summarised in Table II, and those for one experiment given in full in Table III. In order to make the results more comparable the amount of each constituent excreted during the half hour immediately following the exercise has been calculated and expressed as a percentage of the amount excreted during a normal half hour. Occasionally samples could not be obtained exactly at the time desired. For instance no sample of urine could be obtained from H K B O for 7 minutes after running. The urine secreted during these 7 minutes was therefore mixed in the bladder with urine secreted during the 14 minutes before and during his exercise. In estimating the amount excreted during these 7 minutes it has been assumed that the rate of excretion was constant over the whole period. This is certainly an untrue assumption and the error thus introduced tends to diminish the figure representing the change in rate of excretion, but in most cases probably by no very great amount.

As a result of the increased rate of phosphate excretion a certain amount of phosphate has been lost by the body during the three quarters of an hour subsequent to the exercise over and above the amount normally excreted. Because of the bearing this has upon the fall in blood phosphate the amount so lost has been calculated and expressed in milligrams of phosphorus in Table II ("Excess P in urine").

Before discussing possible causes for the rise in blood phosphate we must consider eight experiments done on more prolonged exercise. The results are summarised in Table IV. The exercise took the form of running on the flat for 15 minutes or longer. In Exp 22 the subject was a member of a crew rowing a full course on the Cam. In Exps 20 and 21 samples were taken every seven minutes during half an hour's running. The results seem to indicate a rise during the first 10 or 15 minutes after which the value oscillates irregularly about the higher level for the rest of the run, and then falls rapidly on ceasing the exercise. The rises were, on the average, considerably higher than for the short exercise, giving an average of 25 p.c. against 10 p.c., and taking 10 or 15 minutes to reach equilibrium.

Now the workers on lactic acid in the blood have observed that it is

Table I The "normal" value was obtained by taking the mean of values determined on two samples of blood drawn shortly before the exercise, usually at about twenty minute intervals. In the earlier experiments these two values sometimes varied considerably, the second normally being nearly always lower than the first. The changes due to exercise were always larger than these variations, and in later experiments the difficulty was overcome by the subject's sitting still for at

TABLE I. Short exercise by untrained subjects

Exp.	Subject	Rise per cent.	Fall per cent.	Remarks
1	J B S H.	16	34.5	
2	R E H.	5	24	
3	G A R.	9	29	
4	R E H.	7	10	} Two successive runs with 2 hours interval
		2	19	
5	J T E.	11	22	
6	D B.	8	22	
7	A.C.	12.5	18	
8	R.E.H.	8.1	17.2	
9	J B S H.	22.7	22	
10	J J	11.3	30.8	
Average for men		10.2	22.6	
11	M.M.	10	26	Exps. on three women
12.	B E H.	11	9	Exercise not very vigorous
13	B E H.	4	13	Exercise more vigorous
14.	C.E.L.	17	2	" very "
15	C.E.L.	6	2	" " "
Average for men and women		10	18.8	

TABLE II.

Exp	Subject	Exercise	Excess P in urine mg	PO ₄ per cent.	NH ₃ per cent.	Cl per cent.	Change in pH
7	A.C.	Short	43	604	—	—	—
8	R E H.	"	34	437	—	—	—
9	J B S H	"	45	360	221	26.8	—
10	J J	"	40	330	216	26.6	—
17	R.E.H.	Ran 15'	24.4	340	—	—	—
18	R.E.H.	" 20'	47	628	—	—	—
23	G B	" 20'	23.4	307	—	—	—
<i>Trained</i>							
27	H.K.B O	Short	25.6	277	155	54	7.3-6.2
28	E.H.F	"	39.5	300	113	7.6	6.8-5.1
30	R.H B	"	26	455	90	56	6.4-5.0

A few determinations on sulphates and urea showed no very marked changes from normal

least an hour before the commencement of the experiment. This is a precaution it would be well to take in all further work on blood phosphates. The percentage fall is calculated in a similar way from the

and the molecular proportions are one ion of phosphate to about one hundred lactic ions. This great discrepancy cannot be entirely due to the slower diffusion of the phosphate as even when equilibrium is obtained the rise is never more than 1.5 mg of phosphorus per 100 c.c. A probable explanation is furnished by the work of Embden and Lawaczek⁽⁹⁾ who state that after contraction of an isolated frog's muscle, a large part of the inorganic phosphate set free was recombined with glycogen to form more lactacidogen. A similar process assumed in intact human muscles would account for the comparatively small liberation of phosphoric acid observed.

We thought that the rise in blood phosphate might be due to the acidosis following the exercise⁽⁴⁾. In order to test this as far as possible several experiments were done on the effect of a short period of CO₂ acidosis. In two experiments on J.B.S.H. and R.E.H., lasting 20 and 30 minutes respectively, a rise of 10 p.c. was observed in the blood phosphate. In two shorter experiments on the same subjects no significant increase was observed. It is obvious that the rise of phosphate produced by CO₂ acidosis is a much slower process than that produced by exercise, which appears immediately and seems to reach equilibrium in about a quarter of an hour. 6-8 per cent CO₂ was breathed in these cases.

It remains possible that the intense acidosis produced locally in the muscles may play a considerable part in the raising of blood phosphate, but the probability is that the major part is produced by the breakdown of lactacidogen.

The subsequent sudden fall below normal is more difficult to explain. The most natural place to look for an explanation is in the direction of the sudden increased phosphate excretion observed immediately after exercise. In Exp. 9, as much as 45 milligrams of phosphorus in excess of the normal excretion was lost by the body within three quarters of an hour after running, and at first sight it seems probable that this might account for the sudden drop in blood phosphate. There are, however, one or two considerations that make it unlikely that this is the sole cause.

In the first place the amount excreted is hardly sufficient to account for the drop observed. In Exp. 9 for instance, even if we assume that all the excreted phosphate comes from the blood and none from the lymph and tissues, the 45 mg. excreted would not account for the drop observed, a drop in this experiment from a maximum of 4.6 mg. P per 100 c.c. blood to a minimum of 2.9 mg. That is to say, the blood lost 1.7 mg. P per 100 c.c. and assuming that J.B.S.H. has only 4 litres of blood—

TABLE III. Exp 27 H.K.B.O Ran stairs 9 times 109-116 mins.

Time of collection mins	Vol	pH	Water rate	% P Mg/100 c c	Rate P Mg/hr	Rate NH ₃ MgN/hr	Rate Cl Mille Mols. per hr	Rate urea grams per hr	Rate sulphate Mg per hr
31	202	7.2	391	2.7	10.6	27.5	21.7	1.66	—
57	524	7.4	1210	0.733	8.9	28.3	26.0	1.09	94.6
81	518	7.2	1270	0.840	10.6	31.3	29.2	1.21	96.6
102	225	6.9	643	3.28	21.1	25.5	12.9	0.77	58.2
112	160	6.2	960	9.68	93.0	50.0	7.7	1.05	100.8
125	221	6.3	1020	2.96	30.2	40.0	14.7	1.03	110.9
140	313	6.8	1252	1.15	13.8	44.4	20.0	1.18	117.1
158	398	7.2	1324	0.833	11.0	27.4	25.8	1.07	100.2
177	430	7.0	1357	0.857	11.6	25.9	30.5	0.92	122.8
200	495	6.9	1290	1.075	13.9	—	31.8	1.06	—
210	222	6.8	1330	1.36	18.1	28.5	31.9	1.08	—

TABLE IV

Exp	Subject	State	Exercise	Rise per cent	Fall per cent
16	J.B.S.H.	Untrained	Ran 13	23	17
17	R.E.H.	"	" 15	29.6	16.5
18	R.E.H.	"	" 20'	35	13.8
19	G.A.R.	"	" 8	17	18
20	R.E.H.	"	" 50	15	6
21	R.E.H.	"	" 35'	13	10
22	G.B.	Trained	" 15'	27	0.3
23	G.B.	Rower	" 20	39	7
Mean				24.8	11.1

TABLE V Short exercise by trained subjects

Exp	Subject	Rise per cent	Fall per cent	Remarks
24.	G.B.	5	13.5	A rowing man
25	R.E.H.	7	15	Partly trained
26	W.E.T.	Lost	12	Rugby "Blue"
27	H.K.B.O.	41.5	0	Running "Blue"
28	E.H.F.	10.5	14	Sprinter
29	W.G.O.	5.3	8	Rowing man
30	R.H.B.	12.3	2.8	Running
Mean		11.2	9.3	

after short vigorous exercise that most is liberated (Haldane and Quastel, and Hill, Long and Lupton). The lag of 10 to 15 minutes observed in the production of phosphate would seem to indicate that phosphate ions, if set free within the muscle cell, diffuse out comparatively slowly, taking 10 or 15 minutes to reach equilibrium. Moreover, the increase of phosphate, we observed, is not nearly molecularly equivalent to the increased lactic acid observed by Hill, Long and Lupton in similar forms of exercise(2). Our average figure for short exercise is an increase of 0.3 mg P per 100 c.c. Hill, Long and Lupton observed, in several experiments on "standing running" for 5 to 10 minutes, an increase on the average of about 80 mg of lactic acid per 100 c.c.

short burst of vigorous exercise the sudden call for inorganic phosphate may well play a considerable part in lowering the blood phosphate

Further support to the view that the muscles are directly concerned with these changes is afforded by the fact that there is a marked difference in the phosphate curves given by trained and untrained men. The results of seven experiments on seven different subjects are summarised in Table V. All had been taking regular vigorous exercise of various forms to within a few days of the experiment. It will be seen that in no case was the fall below 15 p.c. and the average was only 9.3 p.c. contrasted with the average fall of 22.6 p.c. for the seven untrained men. The rise on the other hand is substantially the same for both groups, viz., 11.2 p.c. for trained as against 10.2 p.c. for untrained. A point that possibly has some significance is that the rower or the Rugby player gave falls of 13.5 p.c. and 12 p.c. respectively, while the four runners of the University Athletic Club, whose leg muscles were presumably even better trained, gave an average fall of only 6.2 p.c. In attempting to explain the cause of this difference one should note that on the average the trained man excreted less phosphate than the untrained (see Table II), but for reasons specified above it is unlikely that this is responsible for more than a small part of the difference observed. It may be said also that in the trained man the equilibrium is upset less for the same amount of exercise than in the untrained. There was, however, no apparent connection between states of exhaustion and fall in phosphate. In Exp. 10 for instance I.J. was comparatively fresh after the exercise yet gave a fall of over 30 p.c. In Exp. 30 R.H.B. was as exhausted and distressed as any of the untrained subjects yet only gave a fall of 2.8 p.c.

It is then difficult to avoid the conclusion that the untrained muscle absorbs more phosphate from the blood after exercise than the trained muscle. In this connection some experiments by Embden and his co-workers are of interest. Experiments carried out by them on the red and white muscles in rabbits, and on the muscles of summer and winter frogs, all show an increased lactacidogen content in the more active muscle. Moreover, the transition from the low lactacidogen content of the winter state to the high lactacidogen content of the summer state can be brought about very rapidly by bringing frogs from a cold to a warm temperature for a few days (11). It is possible therefore that during the progressively increasing activity of a muscle, brought about by training, the lactacidogen content of the muscle is increased, and if some increase takes place immediately after each burst of exercise by

a very moderate estimate as he weighs 100 kgrs —then he has lost from his blood alone 68 mg of phosphorus. Of this 68 mg only 45 mg or about two-thirds appeared in the urine. In this case then it is quite impossible that the kidneys are responsible for the whole of the fall in blood phosphate. In many other cases it becomes possible only by assuming that all the phosphate excreted comes from the blood which does not in turn draw any from the lymph and tissues. That this is very improbable is shown especially by experiments on CO_2 acidosis. In an experiment in which J B S H remained in the chamber for an hour, it was not until over an hour after the subject had left the chamber that his blood phosphate began to fall. In no case of CO_2 acidosis did it ever fall below normal. Yet during this period the body was losing large quantities of phosphate through the kidneys, producing only a comparatively slow reduction in blood phosphate (Fig 2). This goes on

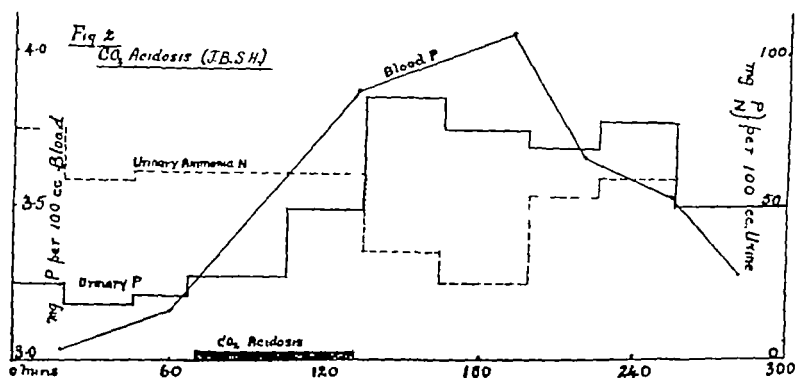


Fig 2.

for some hours after the acidosis has come to an end, showing that there is some reserve the blood can call upon to replenish that lost through the kidneys.

Since then the changes in the blood after exercise cannot be entirely due to the kidney, what other causes may there be? A possible explanation appears when we consider the rapid re-synthesis of lactacidogen from inorganic phosphate and glycogen noted by Embden in many of his experiments. So marked is this phenomenon that it was only when he had seriously depleted the glycogen stores of his animals by starvation and strychnine poisoning that he was able to demonstrate any great lowering of lactacidogen in the muscles after exercise. If a similar process of recovery takes place in man for some time after a sudden

of which the full protocol is given. It will be seen that during the secretion of the sample collected at 180 mins the blood phosphate fell from about 3.50–3.15 mg P p c, and was all the time below the normal level 3.72. Yet the phosphate excretion during this period was at the rate of 82 mg per hour—over four times the normal rate. Similar results have been obtained in other experiments. It may be suggested that this “lag” in the phosphate excretion is not real and is due only to contamination by concentrated urine left behind in the kidney, ureters and incompletely emptied bladder. It is very improbable that this introduces much error, because when, during a normal resting period, the concentration has changed rapidly owing to changes in water rate no corresponding change has been observed in the apparent rate of excretion.

A further cause presents itself on consideration of the work on the reduction in alkali reserve after exercise⁽²⁾ from which it is found that after short vigorous exercise lactic acid was liberated in the blood to an extent sufficient to neutralise a quarter of the alkali reserve, and this did not entirely disappear for nearly an hour. During this period, therefore, there exists a condition of acidosis, practically entirely compensated, similar to that sometimes met with in diabetes. It is well known that in such cases the rate of phosphate and ammonia excretion goes up. Haldane, Hill and Luck, in their experiments on ammonium and calcium chloride ingestion observed the same effect in a compensated acidosis produced experimentally⁽¹²⁾. If the rise of phosphate excretion was in any way due to this cause a similar rise in ammonia excretion would be expected, and, therefore, in several experiments the ammonia excretion was determined. It will be seen in Table II that in the case of two untrained subjects (Exps 9 and 10) the ammonia excretion was more than doubled just after exercise. Among the trained subjects there was in one case (Exp 27) an increase of 50 p c but in the two others no very significant change. It may be noted here that Dautrebande and Davies⁽¹³⁾, in an experiment on exercise, noticed an increase in ammonia nitrogen in the urine collected after the exercise.

In Exps 27, 28 and 30, pH determinations were done on the urine by Miss Watchorn, using Michaelis' method, and in each case a marked fall in pH was observed after exercise (Table II). These observations serve to support the idea that exercise produces a fleeting but profound reduction in the alkali reserve to which the kidneys react vigorously and rapidly by excreting phosphate at a low pH and in some cases by excreting more ammonia. The rapidity with which this fre-

an untrained muscle, then this would account for the greater demand for phosphate by such a muscle. A well-trained muscle presumably already contains a maximum amount of lactacidogen. But it is difficult to account for the anomalous results given by the three women subjects (Table I). One especially, C E L., although she was very exhausted never gave any sign of a fall below normal. The exercise was of the same nature as in the other experiments and was as vigorously carried out. We can only suppose that in this case the re-synthesis mechanism is either absent or works so much slower than normally that it produces no appreciable fall in blood phosphate.

On the whole, however, it seems probable that re-synthesis of lactacidogen plays a conspicuous part in causing the sudden fall below normal observed in the majority of experiments, and that the resulting absorption of phosphate seems to be more extensive in the untrained muscle than the trained.

Phosphate excretion

Embden and Grafe(3) noted an increase in phosphate excretion after prolonged strenuous exercise by well-trained men, and attributed the negative results obtained by former workers to insufficient training and work. As they considered the phosphate output of a "working" day in contrast to a "resting" day it seems probable that the phenomenon they observed is distinct from the one noted by us. Hartmann(11) has also studied the effect of exercise lasting an hour or two hours on the phosphate excretion, collecting the urine in two-hourly samples. He notes a slight inconstant rise in the sample immediately following the exercise, which is followed by a compensating fall in the subsequent sample. It is no doubt the relatively infrequent collection of samples by these workers that masked the great but fleeting increase of phosphate excretion that takes place for a few minutes after short vigorous exercise, for it is frequently followed by a compensating suppression after about an hour. The probable cause of this phenomenon may now be considered.

It is probable that the fleeting rise of the blood phosphate above normal caused a certain amount of increased excretion(8), but it is unlikely that this was the only cause. In the first place the rate of phosphate excretion showed no proportionality to the rise of blood phosphate. In one experiment (No. 7), a comparatively small rise of 12.5 p.c. in the blood phosphate was followed by an increase of over 500 p.c. in the phosphate excretion within the next half hour. More conclusive evidence that another factor is at work is given by Exp. 8,

Figures by Dautrebande and Davies⁽¹³⁾ also show the same effect and it has been noted by others that the excretion of phosphate and chloride show an inverse relation¹. This alone seems hardly sufficient to explain the results obtained after exercise as during the CO₂ acidosis on R.E.H. there was no significant change in the chloride excretion, although the phosphate excretion was more than doubled. We feel the cause of the phenomenon must be left undecided until further work has been done.

SUMMARY

1 It is shown that after short vigorous unaccustomed exercise the inorganic phosphate in blood first rises a little above, then falls considerably below, the normal value in men.

2 In men in athletic training and taking exercise regularly this fall below normal is much less marked.

3 There is a sudden fleeting but very marked increase of inorganic phosphate excretion for about three quarters of an hour after short vigorous exercise, followed in some cases by suppression.

4 There is a less constant but occasionally very marked increase of ammonia excretion.

5 There is a constant very marked suppression of chloride excretion during the same period.

6 It is thought probable that the changes observed in blood phosphate are due mainly to changes in the lactacidogen content of the muscles. The changes observed in the urine seem to be due mainly to the fleeting acidosis caused by the lactic acid formed.

We wish to acknowledge gratefully much helpful criticism and advice from Mr J. B. S. Haldane and Sir F. G. Hopkins, and also the assistance given to us by our experimental subjects.

One of us (R.E.H.) is indebted to the Department of Scientific and Industrial Research for a grant held during the course of the research.

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¹ The literature has been reviewed and further observations made on the isolated kidney by Eichholtz and Starling (17).

quently takes place over short periods is remarkable In 20 minutes A.C (Exp 7) excreted 33 l mg of phosphorus, a rate over eight times his normal

In this connection should be mentioned two experiments on urine secreted during and after prolonged CO_2 acidosis by J B S H and R E H In both cases the phosphate excretion went up to three or four times its normal rate The ammonia, however, showed a slight tendency to drop and certainly no indication of a rise This might be expected as the acidosis was not of a type that involves a great excess of anions which have to be removed (as when the blood is flooded with lactic ions) The increased phosphate excretion follows closely the blood phosphate curve and is probably in this case due to this factor alone (cf Wigglesworth and Woodrow⁽⁹⁾) The results are not in agreement with those found by Davies, Haldane and Kennaway⁽¹⁴⁾, who state that the ammonia excretion in two experiments on H W D went up after CO_2 acidosis

In view of these marked changes observed in the behaviour of the kidney after exercise it became interesting to investigate other constituents in the urine Determinations were done in several experiments of chlorides, sulphates, and urea Two experiments on urea gave 102 p c and 105 p c respectively of the normal rate This leaves out of account the fall in rate observed in all cases *during* the actual running, which has been noted by many observers and put down to the effect of vasoconstriction⁽¹⁵⁾ Only one complete experiment was done on sulphates, and this gave a value of 116 p c but no extreme changes in rate were noted in another incomplete experiment On the other hand in the five experiments in which the chlorides were estimated they showed a profound suppression (Table II) At most they were only a little over half the normal value and in one case (28) only 7.6 p c of the normal This cannot be due to the general suppression of kidney function due to exercise⁽¹⁵⁾ as during the period from which these figures were calculated there was greatly increased phosphate and ammonia excretion and normal urea and sulphate excretion It is improbable also that it is due to such causes as sweating⁽¹⁶⁾ as the period of exercise is so short, or to some shift of chloride ions into the cells due to the acidosis, reducing the plasma chloride because although Dautrebande and Davies⁽¹³⁾ confirmed the increase of chlorides in the cells they found no diminution of the plasma chlorides after short severe exercise Embden and Grafe noted a fall in chloride excretion accompanying the increased phosphate excretion following prolonged exercise⁽³⁾

THE IMPULSES PRODUCED BY SENSORY NERVE ENDINGS Part I By E D ADRIAN

(From the Physiological Laboratory, Cambridge)

THIS paper describes an amplifier used in conjunction with the capillary electrometer and some preliminary observations with it on the action currents set up in sensory nerve fibres by appropriate stimulation of their end organs

Since the introduction of the triode valve a number of workers have used valve amplification in conjunction with the string galvanometer for recording electric responses of very small intensity. The results of Forbes⁽¹⁾ and his co-workers and of Gasser and Newcomer⁽²⁾ have shown how valuable such a combination may be for studying reflex effects in nerve. There is one serious limitation, however, in the ordinary type of string galvanometer which no amount of amplification can overcome, and that is the limitation imposed by the inertia of the moving system. Owing to the mass of the string the record of its movement does not give a true picture of the changes of electromotive force applied to it and the distortion, though of little account in the record of a muscle action current, is quite enough to obscure the true form of the much briefer response of a nerve fibre. With sufficient amplification this defect might be overcome by the use of a recording system of very high natural period (as in the oscillograph), but it cannot be overcome in the usual type of string galvanometer without very extensive alterations¹. The magnitude of the distortion and the possibility of correcting the records by mathematical analysis have been dealt with very clearly by Erlanger and Gasser⁽³⁾ and more recently by Williams⁽⁴⁾. The ideal instrument for recording nerve action currents is undoubtedly the cathode ray oscillograph devised by Erlanger and Gasser, for in this the moving system is a stream of cathode rays, the inertia of which is completely negligible. At present, however, the intensity of the illumination from the ray is far too small to allow photographs to be made from a single excursion, and similar excursions must be repeated many times over before the plate or the eye is affected. As a result the cathode ray oscillo-

¹ These objections would not of course apply to the beautiful instrument recently constructed by Prof. F. A. H. van der Horst, where the string moves in a vacuum and has a very high natural period.

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equation is approximately correct for a properly constructed electrometer but it is naturally no more than an approximation for it leaves the acceleration term $\left(M \frac{d^2y}{dt^2} \right)$ out of account altogether and assumes that on the application of a P.D. the mercury will pass instantaneously from a state of complete rest to a state of motion beginning with the maximum velocity and slowing down as the distance travelled (y) increases. Actually the mercury must take a finite time in accelerating before the maximum velocity is reached and the equation cannot be used with confidence unless we are sure that this time is very small in comparison with the time relations of the potential changes under investigation. How far this condition is complied with by a given capillary can be tested very simply by producing an instantaneous change of potential and recording the movement of the mercury on a rapidly moving plate. Fig. 3 F shows such a record taken on a plate moving 80 cm per second. A large change of potential has been established and the initial velocity of movement is considerable but there is no sign of any rounding of the curve when the movement begins—no sign that is of a progressive acceleration before the maximum velocity is reached. Since the plate travels 8 mm in 0.01 sec it is clear that the mercury passes from rest to its maximum velocity in much less than $\frac{1}{1000}$ sec and a careful inspection of this and similar records shows that the time required for accelerating is probably less than 0.001 sec. Thus the equation (3) may be taken as correct even when we employ the electrometer to record changes such as nerve action currents which are over in a few thousandths of a second.

This freedom from distortion by inertia has been insisted on because it enables the capillary electrometer photograph to be analysed without difficulty and so to give a much truer record of nerve action currents than that obtained with the usual form of string galvanometer. There are however two other features of the capillary electrometer which make it specially suitable as a recording apparatus in conjunction with an amplifier. One is that its own resistance is practically infinite so that the changes in the effective resistance of the valve circuit can be recorded without diminution. The other is the great practical advantage that very little harm is done to it if a large potential difference is applied by mistake. When valves are used a chance interference with the input circuit may give rise to a current large enough to break the string of a galvanometer¹ but in the electrometer such a current will merely cause

¹ The string could be protected by a fuse but this would have to be even more delicate than the string.

graph can only be used in experiments where the same sequence of action currents can be repeated over and over again and it is not suitable for recording an irregular series of action currents such as are produced by the activity of the central nervous system. Another instrument in which the inertia factor is extremely small is the capillary electrometer. This has fallen out of favour with the majority of physiologists because its records need analysis and because of its low sensitivity compared with that of the string galvanometer. These objections have now become of little importance. With the advent of reliable valve amplifiers a low sensitivity in the recording instrument is no drawback at all, and the analysis of capillary electrometer records can be made in a few moments by the machine designed by Keith Lucas⁽⁵⁾. As will be seen, the combination of valve amplifier and electrometer gives us an instrument of such range and precision that it promises access to fields of investigation which are as yet almost unexplored.

The chief point in which the capillary electrometer has the advantage over the string galvanometer lies in the fact that its records show practically no distortion caused by the inertia of the moving system. In both classes of instrument the moving system has appreciable mass and the movement in both must satisfy the usual equation for a damped oscillation,

$$Cy + D \frac{dy}{dt} + M \frac{d^2y}{dt^2} = f(t), \quad (1)$$

where C is the restoring force, and D the damping.

In the capillary electrometer, however, both damping and restoring forces are very large and the third term $M \frac{d^2y}{dt^2}$ is so small in comparison that it is practically negligible. The mercury therefore moves in accordance with an equation which is approximately

$$Cy + D \frac{dy}{dt} = f(t), \quad (2)$$

where f is the displacing force (in this case the applied P.D.) at the time t . This equation resolves itself into the well-known formula for the correction of capillary electrometer records,

$$E_{(t)} = y + h \frac{dy}{dt} \quad \text{or} \quad E_{(t)} = y + k \tan \theta, \quad (3)$$

where y is the vertical distance travelled by the mercury at the time t and the angle θ gives the slope of the curve at that moment. Thus the true value of the potential difference at any moment can be calculated without difficulty.

The work of Burch, Gildemeister and others has shown that this

which contains the high and low tension batteries and is completely sheathed in lead. The input wire is carried in a metal tube which is con-

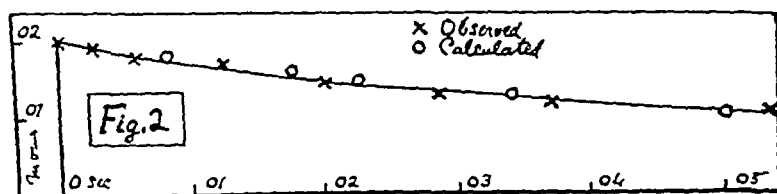
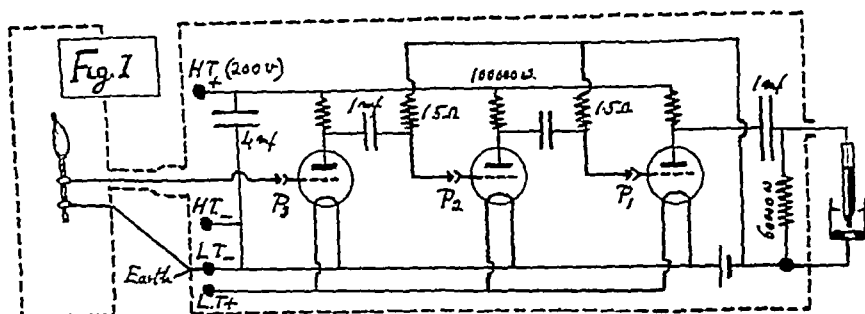


Fig 1 Connections of preparation, amplifier and electrometer Metallic shielding indicated by dotted line The batteries are shielded with the amplifier

Fig 2 Calibration curve Observed and calculated points on applying a steady p.d. of 0.2 millivolts through 50,000 ohms (three valves)

nected with the sheath of the amplifier case. The length of the input wire is about 2 ft. The preparation itself is contained in a large box of sheet iron $\frac{1}{8}$ " thick, measuring $24" \times 12" \times 12"$ and just large enough to hold a cat or rabbit (it might have been made larger with advantage). The sides of the box are hinged and open downwards to allow access to the preparation and the box is placed on a heated animal table. A carbon lamp inside the box aids the rather inefficient heating when warm-blooded preparations are used. The sheath of the input wire is in metallic connection with the wall of the box. With this arrangement it will be seen that the whole system, preparation, input wire and valves are completely enclosed in a continuous metal sheath which is earthed to the water pipes of the laboratory.

This electromagnetic shielding is all that can be desired, for it is possible to run small d c motors 7 or 8 ft from the amplifier without causing any disturbance, and the sparking of an electrically driven tuning fork 11 ft away had no appreciable effect on the record. The

the mercury to run out of the end of the capillary tube, or at the worst will produce some electrolysis and bubbles of gas which are easily cleared by flushing the tube out with mercury. With the present instrument it has never been necessary to take down the tube since the amplifier was installed.

A Description of Instrument

The electrometer used in the present work needs no description, for it is the original instrument designed by Keith Lucas (6) and described by him in this *Journal*. The only changes which have been made are (1) the substitution of a small clockwork feed arc lamp with a choking coil in series to give a very steady illumination and (2) the substitution in the later experiments of a spring time marker in place of the electrically driven tuning fork. The contacts of the fork sparked considerably and although the records showed no sign of disturbance from this it seemed better to use a time marker which should be above suspicion. This consists of a small strip of clock spring fixed at one end in a massive stand. The other end is bent down and held in a catch released by the same movement which releases the photographic plate. The length of the strip is adjusted so that it makes 100 vibrations per second and it continues to vibrate for 2-3 seconds after it has been released. The capillary tube at present in use has a diameter of 0.3 mm at its working part and a pressure of 14 cm Hg is needed to bring the mercury to this part.

The amplifier owes much to the great kindness of Prof. Gasser, who supplied me with details of the amplifier used by him in America, and to the staff of Messrs W. G. Pye and Co. of Cambridge, who redesigned an instrument on the same general lines and planned the very compact and well shielded lay-out of the apparatus. It is a three valve resistance-capacity coupled instrument made on conventional lines but designed so as to be as free as possible from extraneous mechanical and electrical disturbances. The arrangement of the circuits is shown in Fig. 1, and it will be seen that only one high tension and one low tension filament battery is used for all three valves. A single switch in the filament circuit turns the amplifier on or off and the change from one to two or three valves is made in a moment by plugging in P_2 and P_1 . A 4 M.F. condenser is placed across the terminals of the high tension battery (dry cells) to diminish fluctuations in E.M.F. and the resistances are wire wound and shielded in brass cases. The three valves with condensers and resistances are mounted on an ebonite base and contained in a sheet iron box to which the negative side of the filaments are earthed. The box is housed in the top compartment of a large wooden case (38" \times 18" \times 13").

$\frac{1}{1000}$ sec The resistance of the circuit in which this P D is measured may be as high as 500,000 ohms, though the base line becomes unsteady if the resistance is much greater

Naturally such a change could not be detected except on an extremely steady base line and when viewed by eye the shadow of the mercury column often shows irregular oscillations of a few mm. amplitude Fortunately these movements are all extremely slow compared with such rapid changes as a nerve action current, their periods are of the order of $\frac{1}{10}$ to $\frac{1}{2}$ sec or longer and over any given period of $\frac{1}{10}$ sec the base line remains extremely steady provided that the resistance in the input circuit is not too great (cf Fig 3 A and F and Fig 8 C and F)

Distortion How far may we assume that the change of potential in the electrometer circuit is an exact reproduction, amplified a thousand-fold or more of the change of potential in the input circuit? There are two groups of factors which will cause distortion, one of them important only with very rapid and the other with very slow changes of potential If the potential in the input circuit alternated at a period of a million times a second an amplifier with wire wound resistances would be unsuitable The capacities of such resistances are not negligible and at such high frequencies the capacity between the ends of the coil would provide an alternative path for the current and so diminish the impedance of the circuit Thus the amplification would be much smaller for a high frequency than for a low In the present case, however we are concerned with frequencies of the order of a thousand a second instead of a million With the instrument described the amplification only begins to show perceptible falling off when the input frequency is raised to 5000 a second and therefore a series of nerve action currents is well within its powers The other factor which may cause distortion depends on the use of condenser coupling between the valves and on the condenser which intervenes between the output and the capillary These condensers will transmit rapid fluctuations of potential without distortion, but a slow or a permanent change of potential would soon cease to have any effect as the condenser would have time to come into equilibrium by charging or discharging itself The time taken to charge or discharge is proportional to the capacity of the condenser multiplied by the resistance in the circuit For the intervalve condenser this resistance is very large (1.5 Ω) and the rate of discharge is very slow but in the circuit of the output condenser the resistance is only 60,000 ohms plus the effective resistance of the valve (about 20,000 ohms) If a change of potential is suddenly established between the two sides of this con-

complete enclosure of the preparation is usually unnecessary and one of the sides of the box is generally left open during an experiment for convenience in manipulation

Mechanical Shielding Each valve socket is attached to the vulcanite base plate by a small flexible rubber support. The base plate rests on rubber sponges in the metal case which is packed into the outer wooden box with a thick jacket of felt. The wooden box is double walled and consists of an inner and outer case of three-ply wood with cotton waste packed between them, the outside is covered with $\frac{1}{16}$ " sheet lead and the whole stands on four large rubber feet. The front of the box forms a door which opens to allow access to the valves and batteries.

If the door of the outer box and the door of the small inner valve chamber are both thrown open there is enough mechanical vibration of the valve elements to produce a considerable effect on the electrometer when three valves are used. This shows itself as a regular oscillation at about 260 per sec with slower beats superimposed on it. A loud noise near the valves increases the oscillations enormously. When both doors are shut there is no sign of any disturbance unless the box is actually tapped and noises of ordinary volume may be made near it with complete impunity.

Connection to Electrometer The high tension battery produces a large permanent E M F between the filament and plate of the final valve and on this are superimposed the fluctuations which are the amplified image of those in the input circuit. To guard the electrometer from the effect of this steady high potential a 1 M F condenser is placed across the output circuit (Fig 1). The electrometer itself is shunted through a resistance of 60,000 ohms as the damping of its movement depends *inter alia* on the resistance in the circuit and this value gives good results with the particular tube in use.

Amplification The valves used are Marconi D E 5b type, and the voltage amplification with three valves is 1850. With two valves it is 170, but all three valves have always been used in the present work.

When no valves are used the capillary gives a steady deflection of 45 mm on the screen (magnification = 490) for a potential change of 19 millivolts and with all three valves the same deflection would be given by 0.105 millivolts. The rate of movement under this potential is such that a distinct excursion would be produced on a plate moving at 1 metre a second, even though the potential was applied for $\frac{1}{1000}$ sec only. Thus with three valves the capillary should be able to detect a change of potential in the input circuit of about 0.1 millivolts lasting for

there has been no trouble from artefacts due to induction shocks, etc. A small D C electric motor is used to run the film camera when a continuous record is to be made, but it is 15 ft away from the preparation box and it has no appreciable effect on the record. When artificial respiration is used the air is taken from the laboratory compressed air supply through a semi-rotary valve operated by a wind-screen wiper¹ also driven by compressed air.

It is essential that the nerve whose action currents are recorded should not move relative to the electrodes when a record is taken and to ensure this it is usually looped over a small glass hook between the electrodes and the animal (or muscle) with enough slack to prevent a slight movement from pulling on the nerve beyond the hook. The electrodes are plugged with gelatin with a short piece of worsted protruding from the lower end and the nerve is supported by small glass hooks fused to the lower end of the electrode tubes. The tube is filled with Ringer's fluid above the gelatin and the current is led off by a silver wire coated with silver chloride.

Since the electrical effects which are recorded are all extremely small it is essential to make numerous control observations to guard against artefacts. As a routine at the end of each experiment the nerve is killed by crushing or burning between the electrodes and the animal, and a record is made to see if any disturbance can be detected in the electrometer. Almost invariably the base line remains quite steady in these controls. More elaborate control observations will be described in detail later.

B Afferent impulses from Muscles Responses produced by stretching

The experiments to be discussed are to some extent preliminary. They suffice to show the capabilities of the instrument but an exhaustive analysis must be left to a later date.

The most complete observations have been made on the sensory impulses produced by the stretching of a muscle. Sherrington's earlier work and its recent extension by Liddell and Sherrington(8) has made it clear that sensory impulses must travel up to the central nervous system when certain muscles are stretched. In 1921 de Meyer(9) reported very small oscillations observed with a string galvanometer in the nerve when the muscle which it supplies is stretched, and quite recently Forbes, Campbell and Williams(10) were able to show

¹ This is a Lucas screen wiper as fitted on Morris Cars. Very few alterations are needed to drive it by compressed air instead of suction.

denser there will be a transient current in the resistance R which will start at its maximum and should decline to half its initial value in 0.55 sec. The electrometer measures the difference of potential between the ends of the resistance and the change which is recorded will therefore decline at the same rate as the current through R . Fig. 2 shows an analysis of the electrometer record when a P.D. of $\frac{1}{60}$ mv is suddenly established in the input circuit together with a series of points calculated on the assumption that the decline of the recorded potential is due entirely to the output condenser and that the effect of the intervalve condensers may be neglected. The agreement between observed and calculated points is close enough to justify this assumption and the record shows that in $\frac{1}{100}$ sec the observed P.D. has fallen to about 85 p.c. of its initial value. Since the changes which we are concerned with are generally over in less than $\frac{1}{200}$ sec the amount of distortion from this cause will be negligibly small. If it were desired to record the action currents of a muscle instead of a nerve, the distortion would be more important, but it could be reduced easily enough by using an output condenser with a capacity greater than 1 m.f. The only advantage of a condenser of this capacity is its small size and high resistance and the fact that it does not take long to become charged when the amplifier is turned on.

The only other considerable source of error is that introduced in the analysis of the electrometer records in accordance with the equation $E_{(t)} = y + h \tan \theta$. The mechanical analyser designed by Keith Lucas enables this to be done with great accuracy provided only that the photographic image is sharp enough. The essential operation consists in turning the eyepiece of a microscope until the cross wires in it are tangential to the curve traced out by the shadow on the plate. This cannot be done if the image is badly focussed or the negative thin, and for this reason the accuracy of the final result is probably determined more by the quality of the photographic technique than by any distortion in the amplifier or electrometer. Some idea of the accuracy of the analysis under favourable conditions may be gained from records previously published in which condenser discharges of known time relations were photographed with the electrometer and the calculated and observed forms were compared (7).

General points in technique

Up to the present the apparatus has not been used in any experiment which involves the electrical stimulation of the preparation, so that

muscle is stretched and the control with the nerve killed shows no oscillations whatever the weight hanging on the muscle.

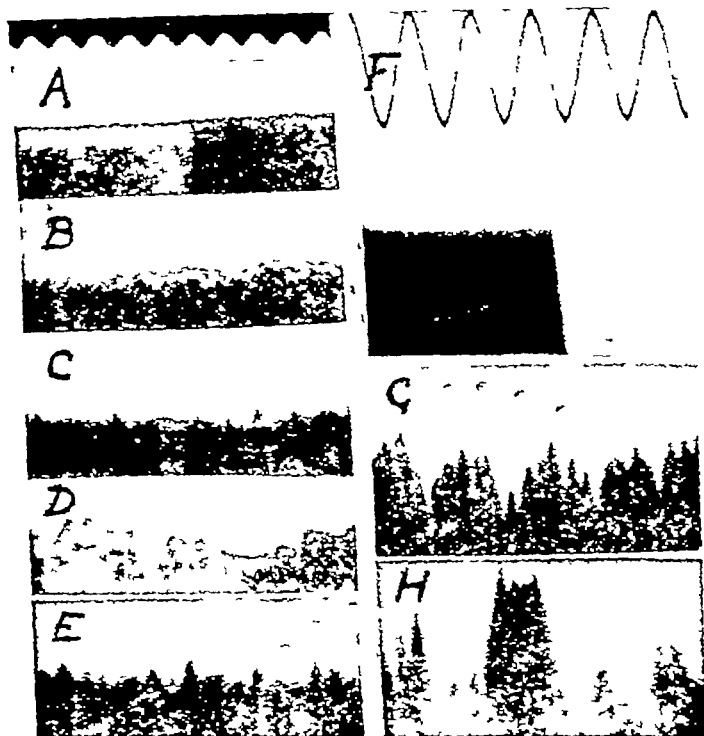


Fig 3 A-D, C and H records of action currents in frog's sciatic nerve on stretching gastrocnemius by a weight.

- A. Control. Nerve killed near muscle. Tuning fork gives 200 d v per sec.
- B. Nerve uninjured, muscle relaxed.
- C. Weight of 10 gms for 10 secs. Nerve uninjured (diphasic).
- D. Weight of 10 gms for 20 secs. Nerve injured between leads (monophasic).
- E. Weight of 100 gms for 10 secs. Nerve uninjured.
- C. Another preparation. 40 gms for 5 secs. on gastrocnemius
- H. Same as G. 40 gms on gastrocnemius and on tibialis anticus
- F. Calibration curve. 0.1 millivolts 3 valves showing instant acceleration of mercury. Spring time marker gives 100 d v per sec

Diphasic and Monophasic Responses The first criterion will be satisfied if we can show that an isolated oscillation is a diphasic change with the first phase indicating negativity at the electrode nearest the muscle and if we can convert this into a monophasic change by killing the nerve between the electrodes. When the tension on the muscle is

conclusively that the act of stretching does produce true action currents which travel away from the muscle towards the central nervous system. In Forbes' work the muscle was jerked suddenly by a spring or made to twitch by an induction shock to the nerve and the action currents were recorded with a string galvanometer with or without a single valve amplifier. His records show a group of three or four oscillations diminishing rapidly in amplitude and he was able to prove quite clearly that each oscillation must represent a group of action currents arising in the muscle and having time relations which do not differ greatly from those of the action currents set up by an electric stimulus. This observation is of fundamental importance as the first definite measurement of the action currents set up in proprioceptor fibres by stimulation of their end organs. The procedure of stretching the muscle suddenly has the advantage of stimulating a large number of the receptors more or less simultaneously so that reasonably large action currents appear in the nerve. With the present apparatus these would be needlessly large and the method of stimulation usually employed consists in stretching the muscle by hanging a small weight on the thread attached to the tendon. A frog's sciatic-gastrocnemius preparation is dissected out and placed on an insulated stand in the metal preparation box with the knee joint held firmly in a clamp and the nerve resting on the electrodes. The thread from the tendon passes through a small hole in the side of the box and over a light pulley. When the thread is completely slack (the muscle resting on a glass plate) the electrometer record is very nearly quiet, though there are occasional small oscillations which are not present in the control records after the nerve has been killed. If a weight of 10 gms or more is hung on the thread and left in position a record made after 10 secs shows a rapid succession of oscillations (Fig 3). With a heavier weight these are more frequent and many of them are larger.

Controls If these oscillations are to be accepted as true action currents they must satisfy certain criteria. A true electric response should consist of a transient fall of potential which passes rapidly along the nerve, in this case away from the muscle. The time relations of the response and its rate of conduction should not differ much from those of the response set up by stimulating the nerve trunk electrically and they should be prolonged considerably by a fall of temperature. Finally the responses should not appear unless we have reason to suppose that the end organs are being stimulated and they should disappear if the nerve is killed between the electrode and the muscle. This last criterion is certainly obeyed. The oscillations only appear in any number when the

the same size. Whether these really represent the activity of a single nerve fibre or whether they are due to a group of fibres acting in unison is a question which must be left open for the present but they copy one another so closely that we are certainly safe in assuming that their time relations do represent some definite characteristic of the response. Fig. 5

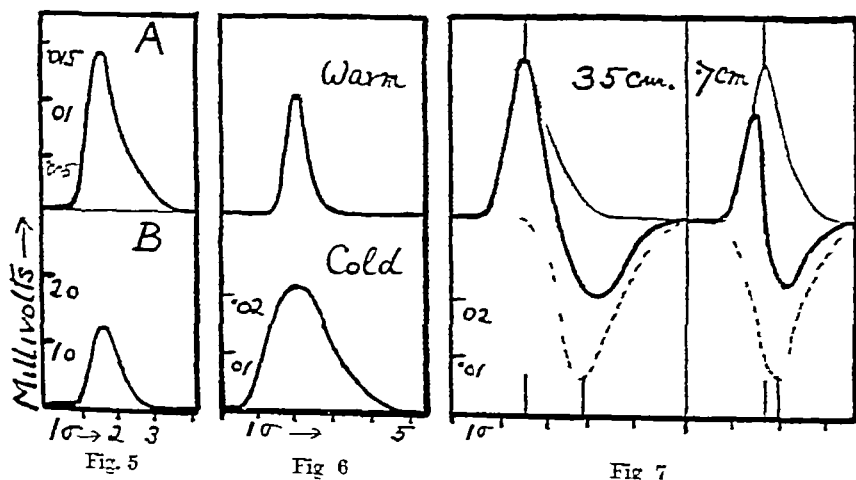


Fig. 5 A—isolated monophasic response produced by stretching muscle
B—response of same sciatic stimulated electrically (no valves)

Fig. 6 Isolated monophasic responses produced by stretching. Nerve warmed and cooled.

Fig. 7 Isolated biphasic responses with varying distance between leads. Thin line gives monophasic response.

gives the analysis of one of these characteristic disturbances on a larger time scale together with the monophasic response produced by stimulating the frog's sciatic with an induction shock and recorded by the same electrometer without amplification. The error in the analysis of the records is fairly large, for with such brief disturbances the whole change only occupies 1–2 mm. on a plate travelling at a metre a second, but the agreement is close enough to justify the statement that the time relations of these responses produced by stretching the muscle do not differ greatly from those of the action current set up by an electric stimulus.

Effects of Temperature The time relations of a true electric response should be prolonged considerably by a fall of temperature. In some previous work (11) a change from 16° to 6° C. was found to prolong the response of a frog's sciatic to about three times its former value. A careful determination of effects of temperature on the present responses has not been made but in one experiment the nerve was alternately warmed by pouring Ringer at 25° over it and cooled by placing some ice

very slight (5 gms weight, *eg*) the oscillations are not continuous but occur as isolated disturbances on a steady base line, and it is then found that the direction of movement of the mercury does show an initial fall of potential at the electrode nearest the muscle. Fig 4 C gives the analysis of a portion of the record in Fig 3 C and it will be seen that each oscillation is diphasic. Fig 3 D and the analysis in Fig 4 D shows the effect

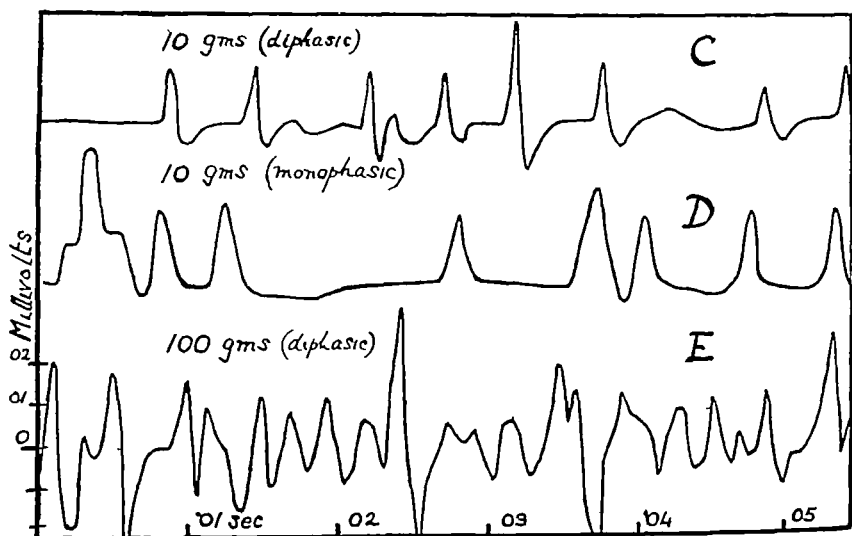


Fig 4 Analysis of records in Fig 3 C, D and E

of killing the nerve between the two electrodes. The electrometer oscillations have a different form and the analysis shows that the response has become monophasic. This change from a diphasic to a monophasic type of response has occurred invariably wherever the nerve is injured between the leads. We have therefore conclusive proof that the disturbances consist of a transient fall of potential passing along the nerve away from the muscle and appearing first under one electrode and then under the other.

Time Relations Some idea of the time relations of the responses may be gathered from Figs 3 and 4. Evidently they are not all of the same duration. The larger responses last on the whole for a longer time and some of them are obviously complex. When the oscillations are crowded together (*eg* Fig 4 E), as they are with a strong stimulus, they vary considerably both in duration and amplitude, but in a record where there are pauses between successive oscillations it is noteworthy that the great majority of them have much the same duration and much

the same size Whether these really represent the activity of a single nerve fibre or whether they are due to a group of fibres acting in unison is a question which must be left open for the present, but they copy one another so closely that we are certainly safe in assuming that their time relations do represent some definite characteristic of the response Fig 5

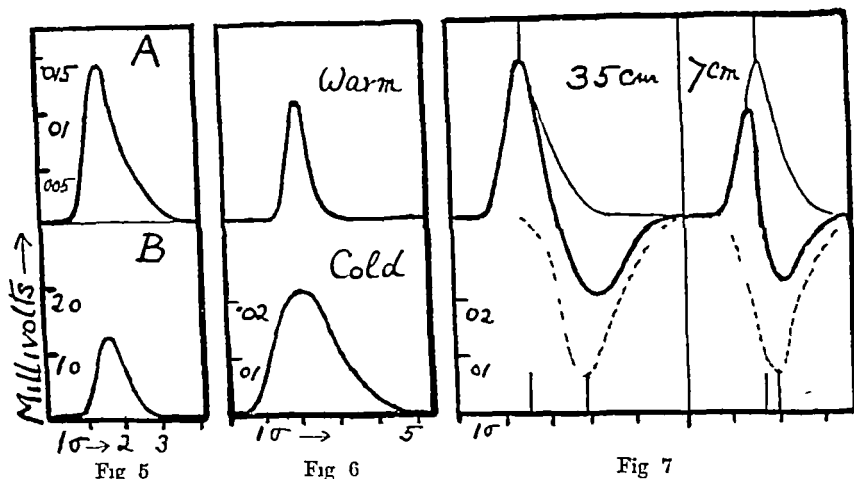


Fig 5 A—isolated monophasic response produced by stretching muscle
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Fig 6 Isolated monophasic responses produced by stretching Nerve warmed and cooled.

Fig 7 Isolated diphasic responses with varying distance between leads Thin line gives monophasic response

gives the analysis of one of these characteristic disturbances on a larger time scale together with the monophasic response produced by stimulating the frog's sciatic with an induction shock and recorded by the same electrometer without amplification The error in the analysis of the records is fairly large, for with such brief disturbances the whole change only occupies 1–2 mm on a plate travelling at a metre a second, but the agreement is close enough to justify the statement that the time relations of these responses produced by stretching the muscle do not differ greatly from those of the action current set up by an electric stimulus

Effects of Temperature The time relations of a true electric response should be prolonged considerably by a fall of temperature In some previous work(11) a change from 16° to 6° C was found to prolong the response of a frog's sciatic to about three times its former value A careful determination of effects of temperature on the present responses has not been made, but in one experiment the nerve was alternately warmed by pouring Ringer at 25° over it and cooled by placing some ice

very slight (5 gms weight, *eg*) the oscillations are not continuous but occur as isolated disturbances on a steady base line, and it is then found that the direction of movement of the mercury does show an initial fall of potential at the electrode nearest the muscle. Fig 4 C gives the analysis of a portion of the record in Fig 3 C and it will be seen that each oscillation is diphasic. Fig 3 D and the analysis in Fig 4 D shows the effect

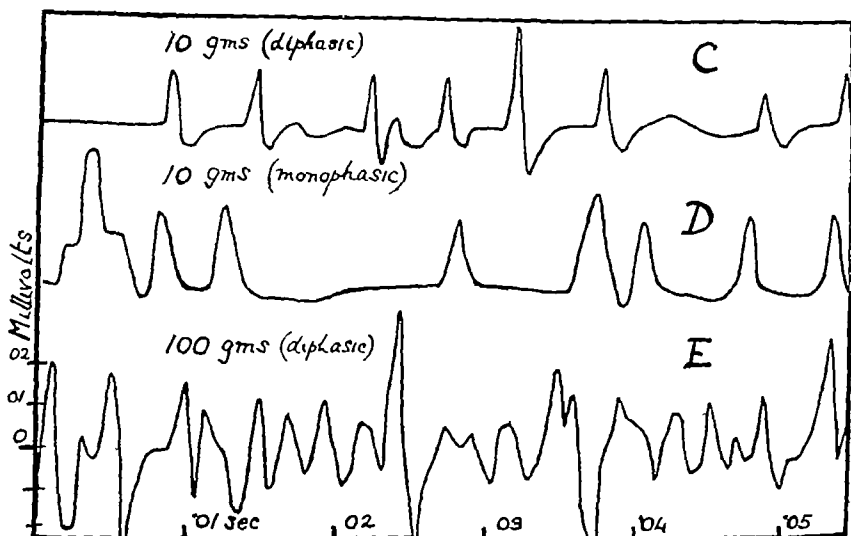


Fig 4 Analysis of records in Fig 3 C D and E

of killing the nerve between the two electrodes. The electrometer oscillations have a different form and the analysis shows that the response has become monophasic. This change from a diphasic to a monophasic type of response has occurred invariably wherever the nerve is injured between the leads. We have therefore conclusive proof that the disturbances consist of a transient fall of potential passing along the nerve away from the muscle and appearing first under one electrode and then under the other.

Time Relations Some idea of the time relations of the responses may be gathered from Figs 3 and 4. Evidently they are not all of the same duration. The larger responses last on the whole for a longer time and some of them are obviously complex. When the oscillations are crowded together (*eg* Fig 4 E), as they are with a strong stimulus, they vary considerably both in duration and amplitude, but in a record where there are pauses between successive oscillations it is noteworthy that the great majority of them have much the same duration and much

the same size Whether these really represent the activity of a single nerve fibre or whether they are due to a group of fibres acting in unison is a question which must be left open for the present but they copy one another so closely that we are certainly safe in assuming that their time relations do represent some definite characteristic of the response Fig 5

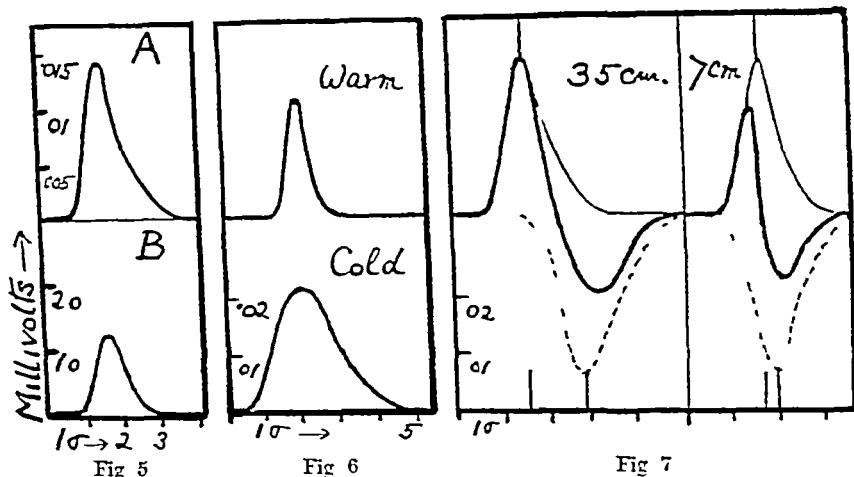


Fig 5 A—isolated monophasic response produced by stretching muscle
B—response of same sciatic stimulated electrically (no valves)

Fig 6 Isolated monophasic responses produced by stretching Nerve warmed and cooled.

Fig 7 Isolated diphasic responses with varying distance between leads Thin line gave monophasic response

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about 1 cm below it, the muscle was stretched by a weight and the monophasic responses were recorded with the amplifier. Fig 6 shows the analysis of typical responses and there is no doubt that the cooling has increased the duration considerably. It is extremely unlikely that an artefact would be affected so much by a fall of temperature.

Rate of Conduction A true electric response in the frog's sciatic should be propagated at a rate somewhere between 20 and 30 metres a second at 15° C. Some idea of the rate of conduction of the present responses may be gained from an analysis of the diphasic curves, though here as in the case of the monophasic curves we are met by the difficulty that the larger excursions may have longer time relations and are sometimes obviously complex. But here too if we confine ourselves to isolated excursions we find a fairly constant interval between the two phases and Fig 7 gives two sets of diphasic curves with different distances between the two electrodes, together with monophasic curves made after the nerve was killed between the electrodes. The rate of conduction works out at 25 m per sec. The possible error is large, perhaps ± 5 metres per sec, but in any case the value is in very good agreement with the known rate of conduction of the nervous impulse in the frog's sciatic.

Frequency The oscillations do not occur regularly, but in any given record the numbers appearing in successive periods of $\frac{1}{2}$ sec are generally very near one another. Now if the oscillations are true action currents their frequency should bear some relation to the state of the end organs, the degree of stimulation, temperature, etc. If they are artefacts depending on the properties of the recording apparatus or on chance disturbances from mechanical or electrical vibrations in the building their frequency would not be affected by the condition of the end organs. The fact that they do not appear at all unless the muscle is under slight tension makes it extremely improbable that they are artefacts and this is confirmed by the fact that their frequency does vary with the degree of tension on the muscle, and if the tension is kept constant with the temperature of the muscle. This is illustrated in the following experiments.

Expt 1 Frog's sciatic gastrocnemius preparation. At room temperature 16.5° C

Stimulus	No. of oscillations (estimated by counting for period of 15 sec.)
(a) Weight of 10 gms. hung on muscle for 10 secs before record was made	210
Weight of 10 gms. on muscle for 20 secs	175
(b) Weight of 50 gms. on muscle for 10 secs	290
Weight of 50 gms. on muscle for 20 secs.	230

Expt 2 Frog's sciatic gastrocnemius Nerve kept at 15° C Temperature of muscle varied.

Stimulus constant		No of oscillations per sec
(a) Muscle at 15° C	Weight of 40 gms for 5 secs	330
(b) Muscle at 5.8° C	Weight of 40 gms for 5 secs	190

Evidently the frequency is determined by something happening in the muscle and not by the mechanical or electrical properties of the recording apparatus

These controls have been dealt with at some length because of the obvious possibility of artefacts in recording changes of potential as small as 0.1 millivolts in a high resistance circuit such as a nerve. They leave no doubt that the oscillations are due to nervous impulses travelling away from the muscle and they show what kind of record we may expect from sensory nerve fibres in general. Granting, then, that we have to deal with true action currents we may proceed to discuss the conditions which give rise to them.

Number of fibres in action The chief difficulty in interpreting these records lies in the fact that we are dealing with a number of afferent fibres and that there is no reason to suppose that they are activated synchronously. It is a point of some importance that the oscillations should appear as clearly as they do, for it is easy to imagine a state of affairs in which the overlapping of impulses in different fibres would be so great that the electrometer record would be smoothed out into a straight line. So far nothing approaching this has been observed in any of the experiments. With a weak stimulus (cf Fig 4 C and D) the oscillations are isolated from one another by considerable pauses. As the strength is increased these pauses disappear and the oscillations are much more irregular in size (Fig 4 E). Evidently there is some overlapping in such records, but even with the strongest stimuli it has not been enough to cut down the average size to any great extent. This must mean that the total number of impulses set up in a given time in all the afferent fibres by a weak stimulus is relatively few—probably not more than 300 in a second, and it is natural to enquire whether the simple, isolated responses such as those in Fig 4 do not each represent the action current of a single nerve fibre. The fact that they all conform closely to a standard size and a standard duration supports this very strongly, for it seems most unlikely that a continuous stimulus such as a state of tension would activate groups of fibres synchronously. The potential change in these isolated responses usually lies between 0.15 and 0.25 millivolts, which is about a thousand times less than the potential change

occurring when the whole sciatic nerve is stimulated electrically. The number of fibres in the frog's sciatic lies between three and four thousand, but it is difficult to say what the relation between potential and number of active fibres would be for such extreme cases as the whole nerve trunk and a single nerve fibre. For the present, then, we must be content with the conclusion that only a very few fibres are concerned in producing the isolated responses—almost certainly less than ten and probably only one.

Effects of change in strength and duration of stimulus This will not be dealt with in any detail on account of the uncertainty in interpreting the results from a nerve containing many afferent fibres. In the near future it should be possible to make use of a preparation containing only one sensory nerve ending, and if this can be done a knowledge of the relation between the stimulus and the frequency of the impulses set up will be far more significant. When many end organs are present, an increase in frequency in the record might be due to an increase in the number of nerve fibres in action, to an increased frequency in each fibre or even to a change from synchronous to asynchronous activity in different fibres. At the same time the results are definite enough and may be stated briefly. In the first place, if the weight is kept constant and is applied at different times before the record is made, it is found that the frequency of the oscillations falls off gradually as the length of the period is increased. This is illustrated in the following experiment.

Expt. Frog's sciatic gastrocnemius preparation. Temp 16° C. Weight of 50 gms hung on tendon. Record made at different times after application of weight.

Duration of application (secs)	No. of oscillations per sec
5	290
10	270
20	165
40	125
80	60
245	47
615	33 (extremely irregular)

It will be seen that some oscillations can still be recorded even though the weight has been hanging on the muscle for ten minutes. Whether they cease immediately on its removal is a point which has not been tested, but they are certainly absent 5 secs later. This persistent discharge of impulses when the tension on the muscle is maintained is in striking agreement with the work of Liddell and Sherrington on the stretch reflex which persists in the same way when the tension is maintained.

The gradual decline in frequency makes it necessary to use a stimulus

of constant duration when changes of strength are investigated. If the period of loading is kept constant (5, 10 or 20 secs as a rule) an increase in the weight causes an increased frequency up to a limiting value of about 400 per second. Thus a weight of 5 gms for 10 secs gave a frequency of 120 per sec and a weight of 50 gms gave 310. More evidence of overlapping occurs as the frequency increases and the record takes on the character of Fig 4 E with a mixture of large and small oscillations. It is an interesting point that in records with smaller weights (or longer durations) where isolated responses can be measured the magnitude of these appears to be constant and shows no signs of varying with the strength of the stimulus. This result is clearly in agreement with the idea of an all or nothing relation between stimulus and nervous impulse.

Afferent impulses from other muscles In the decerebrate cat Liddell and Sherrington find that the stretch reflex is only elicited by tension on the extensor muscles. Tension on the flexors, on the other hand is not without reflex effect, for it inhibits the stretch reflex in the extensors. In the frog tension on the following muscles produces a sustained discharge of afferent impulses in the sciatic gastrocnemius tibialis anticus, sartorius, extensors of the thigh and hamstring muscles. There is no obvious difference between the records from the flexors and those from the extensors. In the spinal cat if electrodes are placed on the popliteal nerve (cut high up in the thigh), tension on the gastrocnemius tendon produces a record almost indistinguishable from those given by the frog's sciatic.

C *Impulses in cutaneous sensory nerves*

The responses so far discussed have all been initiated in the sensory end organs of muscles, i.e. in organs of the proprioceptor class. These are the most easily investigated because the stimulus which excites them is so much more readily controlled and measured than are the stimuli which excite the cutaneous sensory endings. But there is no difficulty in recording the action currents in a cutaneous sensory nerve. The internal saphenous nerve of a spinal (decapitate) cat is dissected out as low down as the knee, ligated and divided high up in the thigh and placed on the electrodes. The leg is allowed to rest on the table, all motor nerves to the limb being divided to prevent reflex movements. A record of the electric effects in the nerve then shows a rapid series of oscillations, and these increase in frequency and amplitude if the skin of the foot is nipped by a pair of artery forceps or pricked by a pin.

(Figs 8 and 10) The oscillations conform to the same tests which were applied in the case of afferent impulses from the muscles. The responses

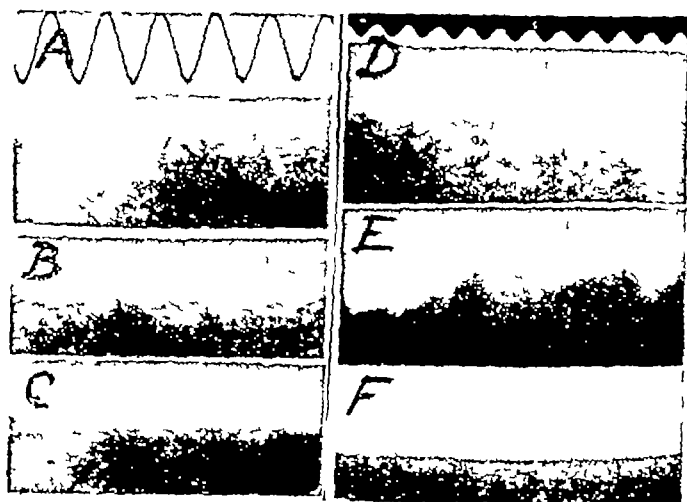


Fig 8 A, B, C responses from vagus of spinal cat. Time marker gives 100 mV per sec

A. Lungs held inflated for 5 secs (diphase)

B. Lungs deflated 5 secs

C. Control. Nerve killed distal to leads

D, E, F responses from internal saphenous nerve of spinal cat. Tuning fork gives 200 mV per sec

D. Skin of leg pinched by forceps. Diphase

E. No added stimulus. Monophasic

F. Control. Nerve killed distal to leads

become monophasic when the nerve is injured between the electrodes and they disappear when the injury is distal to the electrodes, though the injury itself sometimes sets up a rapid discharge of impulses lasting several minutes. A further control consists in leaving the proximal connections of the nerve intact and cutting it distally. The record is then quite free from oscillations though the nerve fibres are uninjured and are in connection with the body of the animal. Records have been made from six nerves and it is noteworthy that in all of these a fairly continuous series of oscillations was present, although the leg was not interfered with in any way. The normal environment seems to contain factors which stimulate the cutaneous receptors, such factors being, no doubt, the pressure of the leg on the table, movements of the hairs produced by air currents, etc. The only added stimuli investigated have been those which would cause pain in the intact animal. The increase in

frequency may be relatively small if the frequency is already high in the resting limb. The maximum rate recorded was 420 per sec, the temperature of the limb surface being 25°C . As the temperature of the nerve was not accurately controlled, not much information can be drawn from the time relations of the responses, but there is no indication that the additional impulses set up by a painful stimulus differ much from those with the limb undisturbed which are presumably due to non-painful stimuli¹.

In two experiments on the spinal cat and one on the frog it has been found that pinching the tendon of a muscle with artery forceps produces a series of oscillations of the usual type in the nerve attached to the muscle.

D *Afferent impulses in the vagus and cardiac depressor*

Observations of the same preliminary character have been made on these nerves, since their sensory end organs belong to the class of visceral receptors and the impulses set up might conceivably differ from those in sensory fibres from the skin or the skeletal muscles. Einthoven⁽¹²⁾ has published string galvanometer records from the peripheral portion of the divided vagus in the dog during the movements of respiration and these show a slow deflection of the string corresponding to the movement of inspiration and a slow return during expiration. On this curve are superimposed a series of more rapid oscillations which are synchronous with the heart beat. The respiratory and the cardiac effects could be separated by leading off from either the vagus or the cardiac depressor in the rabbit. These observations (which confirmed and extended the earlier work of Lewandowski and of Tschermak and Koster) show that the vagus becomes electro-negative during inspiration, as would be expected if afferent impulses were passing up it, but there is nothing to indicate the nature and frequency of these impulses.

The following experiments have been made with spinal (decapitate) and decerebrate cats and with rabbits anaesthetised with urethane. One vagus was divided at the level of the lower border of the thyroid cartilage, and the lower, distal portion was dissected out as far down as the sternum and placed on the electrodes, the distal part of the nerve being looped over a glass hook to prevent the pulsations of the carotid from being transmitted to the electrodes. When continuous records were required the shadow of the capillary was thrown by a train of

¹ Erlanger and Gasser have shown that there are probably differences in the time relations of various classes of sensory impulse, but they are scarcely great enough to be detected in the present records.

mirrors on to the slit of a camera containing cinematograph film driven at a constant rate by an electric motor. The movements of respiration were recorded on the same film by a lever connected by a thread to a flat plate resting on the chest of the animal.

Typical results are shown in Fig 9. Fig 9 A and B are from the

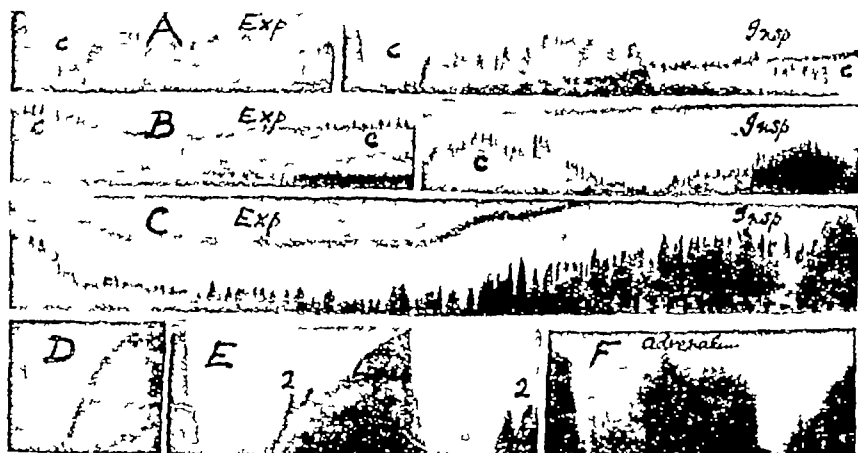


Fig 9 Portions of continuous records on cinematograph film from vagus and cardiac depressor nerves. Time marker (on C) gives 02 sec.

A Spinal cat. Artificial respiration. Record from vagus showing cardiac (c) and respiratory impulses.

B Decerebrate cat. Natural respiration. Cardiac (c) and respiratory impulses.

C Rabbit, urethane. Record from vagus.

D and E Ditto. Record from cardiac depressor.

F Ditto. After injection of adrenalin.

cat and impulses of depressor origin are therefore included. These occur in groups at the same rate as the heart beat and each group is marked *c* in the figure, but their consideration may be deferred as they can be studied more conveniently in the rabbit. The respiratory effect is quite clear. Oscillations occur during expiration as well as inspiration, but their frequency and amplitude is greatest at the height of inspiration and least at expiration. The most striking result is the absence of any sign of a renewed discharge of impulses at the moment when the lungs are most deflated. In the records from the cat there is very little activity in the vagus throughout the period of expiration apart from the groups of cardiac impulses. In the rabbit there are more oscillations during expiration though the increase on expansion of the lungs is clear enough.

This difference may be due to the fact that the breathing was shallower and more rapid in the rabbits, so that the lungs were never as completely relaxed as in the cat, but the oscillations might also be due to impulses in sensory fibres coming from other regions than the lung

Nature of stimulus to vagal endings The increased flow of impulses during inspiration might be due to the actual movement of expansion of the lungs or to the state of tension produced in the tissues, or to both. In a number of experiments the tube from the trachea was clamped and the lungs allowed to remain in the expanded or relaxed state for several seconds before the record was made. Typical records are given in Fig 8. In one of these experiments the lungs were allowed to remain inflated for 20 secs, but the oscillations were as clearly marked as in records made during the actual movement of expansion. Clearly then the state of expansion of the lung is an effective stimulus to the vagal endings and the flow of impulses continues as long as the tension is maintained, just as it does in the afferent fibres from a skeletal muscle. Since there is a steady flow of impulses as long as the lungs remain expanded, the total number of impulses reaching the centre will go on increasing until the lungs are relaxed. If the effect of the impulses on the centre is cumulative, this would account for the fact that the contraction of the expiratory muscles becomes greater and greater as long as the breath is held.

If the lungs of a spinal cat are deflated forcibly and the trachea tube is then clamped, a record taken 2-3 secs after does not show any more oscillations than are present if the chest is merely allowed to deflate itself. Indeed, as far as these records are concerned, there is no evidence that deflation of the lungs is an effective stimulus to the vagal endings.

Nature and Frequency of impulses Analyses of two records are given in Fig 10. The time relations are much the same as those in the cat's internal saphenous, though here too the temperature of the nerve was not accurately controlled. The maximum frequency with the lungs inflated has been 450 for short periods.

The close likeness of the records from the vagus, internal saphenous and from the frog's sciatic suggests either that all the sensory fibres concerned are very much alike, or that the characteristic record is an artefact with oscillations determined by the recording instrument. This possibility has been discussed already and rejected, and if further evidence were needed against it, it is supplied by the records from the cardiac depressor which are of an entirely different character.

E Impulses in the cardiac depressor

The rabbit's cardiac depressor is a very slender nerve made up entirely of afferent fibres from the heart. For this reason the responses in it are large compared with those of a mixed trunk like the vagus or sciatic. Typical electrometer records are given in Fig 9 D, E and F, and an analysis of one complete cardiac cycle in Fig 10. The responses occur

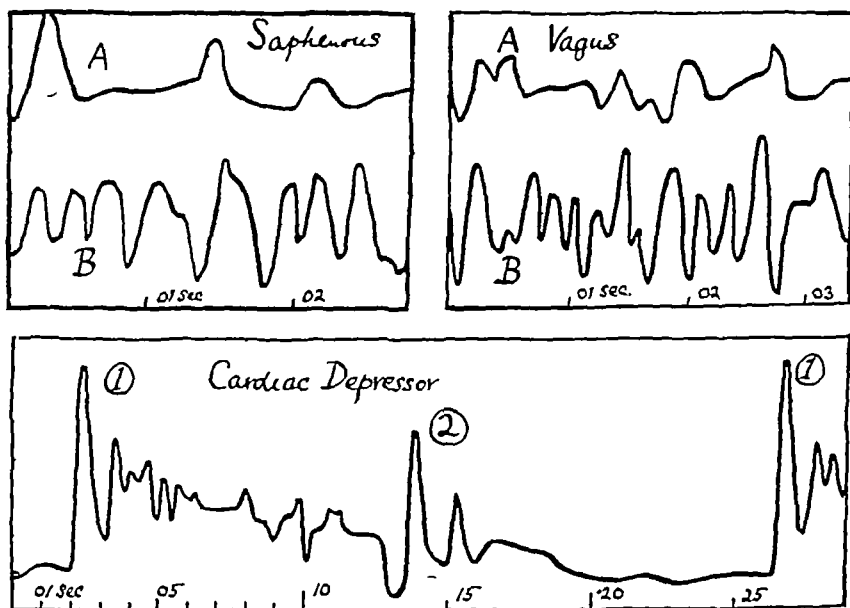


Fig 10 Analysis of records from internal saphenous, vagus and cardiac depressor

Saphenous A. Monophasic, no added stimulus

B. Diphasic, skin of leg pinched.

Vagus (spinal cat) A. Monophasic, lungs deflated 3 secs.

B. Diphasic, lungs inflated 3 secs

Cardiac depressor Rabbit Initial wave marked 1 diastolic 2

in groups synchronising with the heart beat with a distinct pause between successive groups. The pause (presumably diastolic) is broken abruptly by one or more very large oscillations of EMF followed by a rapid succession of smaller oscillations. Before these have died out completely there is nearly always another large oscillation (marked 2 in the figures). After a few more small oscillations the diastolic pause ensues. Using an ordinary galvanometer Tschermak and Koster⁽¹³⁾ found that a negative variation was produced in the nerve by distension of the aorta, and in view of this we may take the first large outburst of

impulses as due to the rise of pressure in the aorta at the beginning of the ventricular systole and the second to the rise of pressure when the aortic valves close. Records from different animals or from the same animal at different times show a great variation in the number of smaller oscillations. The conditions determining this have not been studied but an injection of adrenalin (5 cc of a 0.1 p.c. solution) into the ear vein of the rabbit was made in two experiments and was followed by a great increase in the number of oscillations, the smaller waves continuing throughout the diastolic pause. In the records of the cat's vagus if the decapitate preparation is used the groups of oscillations which show the cardiac and not the respiratory rhythm have much the same character as those in the rabbit's depressor but in the only decerebrate preparation the large initial and "dicrotic" waves are not evident (Fig. 9 B). The obvious need for the correlation of these records with determinations of the blood-pressure makes further discussion premature.

The foregoing results have been put on record more as an indication of the capabilities of the recording instrument than as a contribution to any branch of physiology. All of them suffer from the fact that the preparations employed have contained many afferent fibres and that we do not know how many are in action at a given time. But the instrument is clearly capable of recording the action current of a single nerve fibre even though it is surrounded by a thousand or more inactive fibres in a large nerve trunk, and it should not be a matter of great technical difficulty to isolate a single sensory ending and so to obtain the positive information lacking in the present experiments. Experiments on these lines are in progress and have already reached a great measure of success.

SUMMARY

The paper describes a combination of a capillary electrometer with a three valve amplifier which is capable of recording rapid changes of potential of the order of 0.1 millivolts with almost complete absence of disturbance from mechanical and electrical artefacts. With its aid it has been possible to record the action currents accompanying afferent impulses in the frog's sciatic nerve when the gastrocnemius is stretched by a weight, in the cat's internal saphenous nerve when the skin is pinched, in the cat's and rabbit's vagus when the lungs are inflated and in the cardiac depressor nerve of the rabbit. Numerous control observations have been made to exclude the possibility that the recorded oscillations of potential are due to any other cause than the passage of impulses in the nerve. It is probable that many of the oscillations represent action

currents in a single nerve fibre, and these have the same general form and the same general time relations (allowing for temperature differences) in all the sensory nerves in which they can be isolated sufficiently for measurement. There is no evidence that an increase in the stimulus increases the size of the action currents in single fibres, but the frequency of the impulses in the nerve trunk increases and leads to interference and overlapping of impulses in different fibres. When a muscle is stretched by a weight the discharge of afferent impulses continues for as long as ten minutes, provided that the tension is maintained. Similarly the passage of the impulses up the vagus continues (for as long as 20 secs) if the lungs are held in the expanded state. No evidence was found of any renewed discharge of impulses in the vagus on deflation of the lungs. More detailed analysis of these results is postponed until experiments have been made on preparations containing a known number of sensory endings, if possible only one.

I wish to express my thanks to Miss S. Cooper for her valuable help in some of the earlier experiments.

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ON THE ELASTICITY OF SKELETAL MUSCLES

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In text-books dealing with the physiology of muscles it is common to find a diagram showing the relation between length and load in skeletal muscles in excited and unexcited state respectively. The curves, differing widely when the load is small approach gradually one another and unite when the load has become large enough to prevent shortening of the excited muscle. The curves show clearly that the extensibility of the excited muscle is greater than that of the unexcited one. These diagrams are based mainly on the investigations of E. Weber(1)

Weber determined in a number of muscles "die Ausdehnbarkeit," i.e., the difference in length caused by increasing the load from p to p_1 , divided by the average length of the muscle and by the increase in load, or the increase in length caused by a load of 1 gm within the given interval of length $= 2 \frac{l_1 - l}{l_1 + l} \frac{1}{p_1 - p}$. The experiments show that the excited muscle became more "ausdehnbar" i.e., its coefficient of elasticity decreased. The results of the experiments here concerned were controlled by means of swing-experiments, the technique of which is not mentioned in detail in Weber's paper. It was found that in excited muscles the time of an oscillation increased in spite of the fact that the muscle became shorter as well as thicker than in the unexcited state.

Kaiser(2) adopting a swing-method of Wundt(3) seems to have arrived at similar results. Neither the assumption taken as starting point nor the conclusions of Kaiser are tenable, but he found on frog's sartorius loaded with 21.5 gm. that the time occupied by an oscillation was increased when the muscle shortened in incomplete tetanus, in complete tetanus, however, the time decreased slightly. Judging from the figures published it is justifiable to assume that the last-named result is in accordance with the former, but as no length-determinations are given the figures cannot be interpreted with certainty. Also Wundt obtained quite similar results. Both these authors sometimes found the

time of an oscillation unaltered when the excited muscle was not allowed to shorten, these occasional results may have been due to overloading of the muscle to such a degree that its structure was damaged, thus Kaiser loaded the frog's *gastrocnemius* with 521.5 gm. In other experiments of Kaiser's in which the load was reduced to 221.5 gm. the time occupied by a swing increased when the muscle was stimulated, in spite of the fact that no shortening took place.

Dreser made a series of experiments with increasing load (4). His result confirmed the theory of Weber so far as the elasticity of muscle proved to decrease with increasing load. On the other hand, Dreser's results did not agree with the formula of Wertheim (5) deduced by this author from Weber's experiments supplemented by experiments on dead material by the author himself. The formula of Wertheim, $y^2 = ax^2 + bx$, represents a hyperbola with the top in the point of intersection of the coordinate-axes. Dreser found, however, when calculating results of experiments of his own as well as those from the available literature that the coefficient of x^2 in the formula with increasing load changed its sign, i.e., the curve represented by the equation changed from a hyperbola to an ellipse with the parabola as a transitional state. Dreser supposes that the discrepancies may be explained by assuming that extension of the muscle causes it to contract and thus to counteract the extension, the author often observed fibrillar contractions in the muscles especially in the moment when the extending weight was removed, when the muscle was loaded the conditions of observation were more difficult. Recent investigations of Eddy and Downs (6) seem to support this point of view.

Regarding the differences in shape between the elasticity curves of muscle and those of inorganic material it was shown by Wertheim that in drying muscle b (the coefficient of x in the equation) decreases towards zero, indicating that the curve of elasticity becomes a straight line as in the case of inorganic matter. Dreser is of opinion that the hyperbolic curve of muscle—and of organic matter on the whole—is due to the fact that the muscle consists of numerous elements arranged in such a manner that a small load causes stretch on a single or a few elements only, while a somewhat heavier load affects the whole number of elements. Thus the successive parts of the curve in fact belong to different elastic bodies.

In a series of critical papers Blix (7) deals with our problem, finally giving it up as insoluble. Blix emphasizes that stretch experiments cannot be utilized, because the length of a muscle for a given load is a

function of time, when the muscle is loaded the length may increase for hours or days, and meanwhile the muscle may die or its elasticity may be altered for other reasons. Moreover Blix is aware of the fact that stretching may cause contraction of the muscle, and to a different degree according to the strength and the duration of the stimulus, he has in fact anticipated the recent experiments of Gasser and Hill(s) with quite the same results thirty years ago. Recently Schleier⁽⁹⁾ has experimented on the resting and the contracting muscle by means of a method devised by Marey⁽¹⁰⁾, he finds that the elasticity-coefficient of muscle is less during contraction than it is during rest.

Lastly we have a paper of Gasser and Hill(s) who have arrived at a quite different result regarding the elasticity of muscle. The authors make use of a new method in so far as they measure the damping effect caused by the resting and the contracting muscle on a swinging steel spring. The result is that the damping effect is increased to a very high degree when the muscle is stimulated. Yet it must be born in mind that the swinging spring, the mass of which is enormously much greater than that of the muscle, in each swing extends the muscle, and the method must therefore be regarded as in all essentials a stretch-method, it may thus give rise to some, at least, of the same errors as do the latter methods. The most dangerous error, especially with regard to the method of Gasser and Hill, is that stretching may cause the muscle to contract. If the muscle in the damping experiments is excited by the swinging of the strong steel spring—and it is not easy to see how this could be avoided—the immediate effect will go in the opposite direction of damping, and we have thus opened a way which according to the mutual strength of the opposed forces may damp or prolong the duration of the swinging of the steel spring. It is not clear that the authors have done anything to prevent excitation of the muscle. We cannot, therefore, accept their point of view before due allowance is made for this possible source of error.

In some previous papers two misunderstandings of a more general character are met with. In the paper of Kaiser⁽²⁾ as well as in the monograph of Triepel⁽¹¹⁾ it is maintained that contractility is a special muscular property, the effect of which sometimes acts in the same and sometimes in the opposite direction of that of elasticity, thus rendering the determination of the elasticity of excited muscles difficult or impossible. We want to emphasize that the contracted muscle, hanging in the myograph, as well as the unexcited one, is a physical object, the elasticity-coefficient of which may be determined under the conditions

defined without regard to the fact that this object may possess other physical or physiological properties, in so far as such properties are not influenced by the experimental technique. As Dittler⁽¹²⁾ does not discuss the swing-methods for determining muscular elasticity, pointing out the fact that the skeletal muscle in its natural position is never torquated, we want to emphasize further that the determination of the coefficient of elasticity of muscle is a purely physical problem. When this physical problem is solved the results may be applied to physiological questions.

Most of the papers quoted above lead to the opinion that loading (and especially stretching) stimulates the muscle to contraction, and experiments performed by us according to similar methods point in the same direction. When we want to determine the effect of stimulation upon the elasticity of the resting muscle, it will accordingly be natural to omit as far as possible stretching in any form. We have found it preferable, therefore, to make use of a swing-method and to charge the muscle with only a minimal load.

Method Our experiments have been made on the frog's sartorius. After cautious removal from the freshly killed animal the muscle was fixed in the clamp of a myograph. To the other end of the muscle a swinging device consisting of a swine-bristle at each end bearing a small ball of picene was fixed by means of a frame of very thin brass wire. The weight of the bristle and balls was 0.363 gm, the distance between the centres of the balls 5.2 cm, and the moment of inertia related to a perpendicular axis through the middle of the bristle was approximately $2.45/982$ absolute units. From the brass frame a thread of 0.4 mm thickness dipped into a wide glass tube filled with tap-water, this stand furnished the one pole of a secondary coil while the other pole was represented by the clamp of the myograph. The resistance offered by the water when the thin metal thread was turned round its axis proved to be negligible. Care was taken of course to prevent any movement between the muscle and the swinging device as well as between the mutual parts of the latter. Thus the length of the swinging part of the

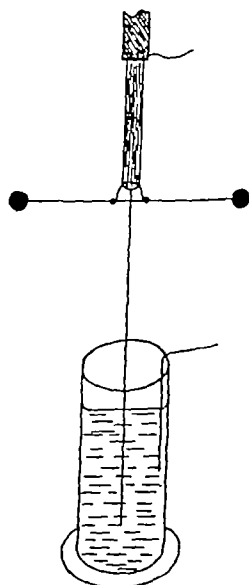


Fig 1

muscle could be measured out exactly between the two lines of fixation by means of a moveable point mounted on a stand bearing a millimetre scale and provided by a worm-gear which permitted of rapid changes of the position of the index. Tenths of a millimetre could be read off. Time was taken by means of two stop-watches, the turning of the balls was observed directly, tenths of a second could be read off and the two readings were averaged. If possible five oscillations were observed but when the muscle could not keep its length constant during so long a period the number was reduced, the time occupied by a single oscillation being always sufficiently long to secure a reliable reading. The whole system was made to oscillate by blowing gently on one of the balls. In each series of experiments the corresponding values of length and period of swing were first determined on the resting muscle in two or more experiments, then a tetanizing current was sent through the muscle and the determinations repeated at various degrees of shortening.

The coefficient of elasticity E may be calculated from the equation

$$(1) \quad t = \frac{I + k}{F} - \frac{16\pi l I}{r^4}$$

In this formula t means the time occupied by one oscillation, I the momentum of inertia, r the radius, and l the length of the swinging body, k is a constant. As, however, more of these magnitudes may be difficult to measure with any certainty, and as the absolute value of the coefficient of elasticity was considered of minor importance as compared with the relative value of the coefficient in the excited and the unexcited state of the muscle, the above formula was modified. As a matter of fact the volume of the muscle is not altered to any appreciable extent during contraction, and hence with a fair approximation we have

$$(2) \quad l_1 l_2 = r_2^2 / r_1^2,$$

where the indices 1 and 2 refer to the resting and to the contracting muscle respectively. If we now introduce these indices in the formula (1), and if in the second of these equations for r_2 we substitute the expression for r_2 derived from (2), then this equation divided by the first gives the formula

$$(3) \quad \frac{E_1}{E_2} = \frac{t_2^2}{t_1^2} \cdot \frac{l_1^3}{l_2^3} = \frac{\alpha_1}{\alpha_2}, \text{ when } \alpha = F/t^2$$

In this formula the figures r , I and k are eliminated, the only figures needed from the experiments are l and t , the corresponding values of which may be ascertained in the manner described above.

In the first two series of experiments (Tables I and II) the muscle was employed in its natural form, as however the formulæ concerned

presuppose cylindrical bodies the edges of the muscle in later experiments (Tables III and IV) were cautiously cut away in such a manner that the central portion of the muscle utilized had a nearly circular cross section

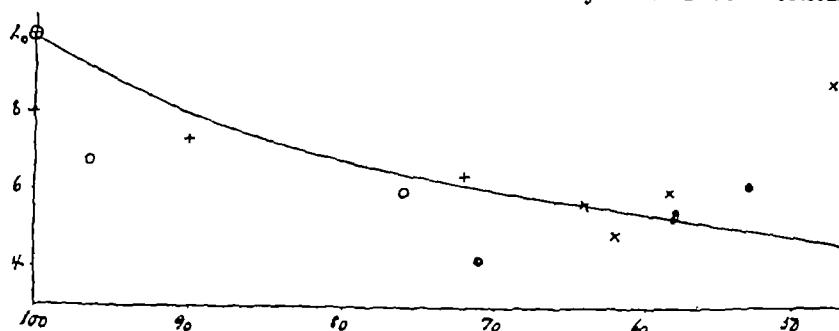


Fig 2 Abscissa = relative length Ordinate = $E_r = E_2/E_1$

Table I

- ⊕ Rest before stimulation
● Stimulation
○ Rest after stimulation

Table II

- ⊕ Rest before stimulation
× Stimulation
+ Rest after stimulation

TABLE I

No of Expt	Length cm	Time for 1 oscill sec	$\alpha = l^2/t$	l in p c of l_{rest}	E_r	Expt conditions
3	2 60	2 68	2 44	100	1	Rest before stimulation
1	1 38	1 32	1 51	53	0 62	Stimulation
1	1 50	1 58	1 35	58	0 55	"
2	1 98	2 30	1 47	76	0 60	Rest after stimulation
4	2 51	3 10	1 65	96 5	0 68	"
1	1 85	2 49	1 02	71	0 42	Stimulation
1	1 50	1 60	1 32	58	0 54	"

TABLE II

4	2 41	3 29	1 29	100	1	Rest before stimulation
3	1 50	2 31	0 63	62	0 49	Stimulation
1	1 74	2 52	0 83	72	0 64	Rest after stimulation
1	2 16	3 27	0 94	90	0 73	"
1	2 42	3 70	1 03	100	0 80	"
2	1 41	1 90	0 78	58 5	0 605	Stimulation
1	1 13	1 13	1 13	47	0 88*	"
1	1 54	2 24	0 73	64	0 57	"

* For unknown reasons obviously fallacious

TABLE III

2	2 47	5 81	4 47	100	1	Rest before stimulation
1	2 50	6 32	3 91	100	0 88	Rest after stimulation
1	1 76	5 50	1 81	71	0 41	Stimulation
1	2 14	6 85	2 09	87	0 47	Rest after stimulation
1	2 29	6 85	2 56	93	0 57	"

TABLE IV

2	1 80	4 03	0 359	100	1	Rest before stimulation
1	0 86	3 40	0 055	48	0 15	Stimulation
1	1 54	4 90	0 152	85 5	0 42	Rest after stimulation
1	1 35	4 45	0 124	75	0 35	Stimulation
1	1 49	3 85	0 223	83	0 62	Rest after stimulation
1	1 64	3 72	0 319	91	0 89	"

The tables and figures show that all the muscles examined behave in principle in the same manner only quantitative differences are found

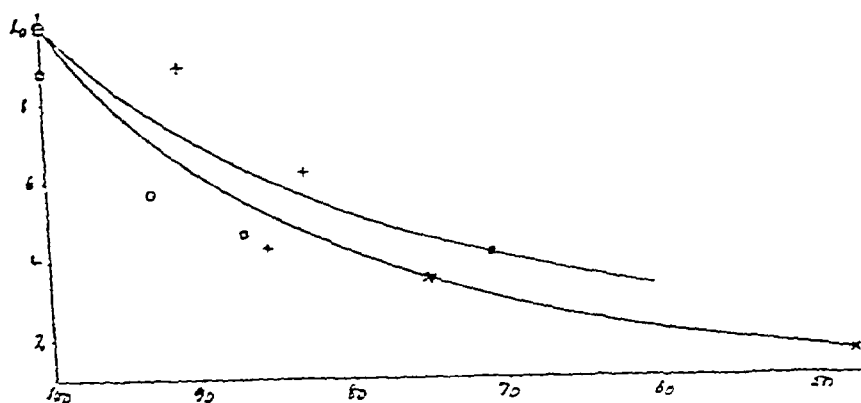


FIG. 3 Ab-scissa = relative length Ordina $r = E_r$

Table III

- Post before stimulation
- Stimulation
- Rest after stimulation.

Table IV

- Post before stimulation
- Stimulation
- Post after stimulation

and it may be noted especially that the changes are most pronounced in the circular muscle-strips. It is evident that the coefficient of elasticity always decreases when the muscle is stimulated. It is further evident that the new elastic state of the muscle caused by excitation does not pass away immediately when stimulation ceases but vanishes gradually as the muscle regains its original length. Yet the two changes do not seem to be causatively united: sometimes the elasticity reaches its original level before the original length is reached and sometimes we find the reverse order of events. It may be noted that the slope of the curves in Figs. 2 and 3 is rather arbitrary owing to the relatively small number of contraction experiments; it is probable that a more extensive material would prove the fall at the beginning of the curve to be somewhat steeper.

It might perhaps be supposed also that the torsion of the swinging muscle might act as a stimulus and give rise to localized contraction. If so the effect would increase with increasing amplitude of the oscillation, the experiments have shown however that no such increase takes place. On the other hand, the stretch on the muscle is influenced also by the load, being less when the load as in our case is very small. We do not believe however that gentle movements going on very slowly

are able to stimulate an unloaded muscle, and moreover it must be remembered that a partial excitation of the resting muscle must change its elastic properties in the direction of those found during contraction and thus tend to diminish the differences found in our experiments. In other words, the difference found by us between the coefficient of elasticity of the muscle in the resting and the excited state is a minimum value.

The viscosity of the muscle has not been taken into account in these investigations, future experiments may decide whether this property is altered by stimulation.

SUMMARY

The relative coefficients of elasticity of the resting and the excited muscle are determined.

The experiments show that excitation of the muscle causes the coefficient of elasticity to decrease. This change takes place rather suddenly while the original resting level is regained gradually and independent of the simultaneous changes in length.

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THE INTERPRETATION OF THE ELECTROMYOGRAM OF STRIATED MUSCLE DURING CONTRACTIONS SET UP BY CENTRAL NERVOUS EXCITATION¹

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STRIPED muscle contracting as a result of impulses from the central nervous system exhibits frequent action currents. For the voluntary contraction of human muscles this was first shown by Piper(1) and Florence Buchanan(2), for the contraction of muscles under decerebrate rigidity by one of us(3, 4), for other different kinds of reflex contractions by Beritoff(5) and for the respiratory contractions of the diaphragm by Dittler and Garten(6, 7). Piper stated that the action currents of human muscle during voluntary contraction show a fairly regular rhythm of 50 oscillations per second. This statement has not been generally accepted. Nearly all observers agree that the action currents of voluntarily contracted muscles are irregular, not rhythmical, and that their frequency is much higher than Piper stated, mostly one finds frequencies recorded of 100-200 per second. About 160 may be regarded as the ordinary average.

There is less agreement as to the origin of these action currents, i.e. whether the action currents led off from the muscle represent a peripheral, muscular rhythm or whether they are of nervous central origin. Buchanan defended the first view, Piper the second. Dittler and Garten(7) established an important fact bearing on the question. Registering electrograms from the diaphragm as well as from the phrenic nerve during the respiratory movements of the animal, they showed that these two curves, the electroneurogram and the electromyogram, were nearly identical. From this fact they concluded that every natural impulse of the phrenic nerve is followed by an excitation of the muscle. Gasser and Newcomer(8) some years ago corroborated this statement. But the interpretation of the rapid oscillations of the electromyogram in contractions of central origin, as the expression of a muscular rhythm

¹ For a fuller account of the experiments, see Dissertation by Dr Brevée, *Electromyographische Onderzoekingen by verschillende vormen van centrale innervatie bij de kat* Utrecht 1925.

put forward for the first time by Buchanan, has also found ardent defenders Forbes and Rappleye(9), repeating the older experiments of Buchanan with warming and cooling of the contracting muscle, found that warming the muscle gave rise to an augmentation of the frequency of the action currents, whereas lowering of the temperature resulted in a depression of the frequency. From this observation they concluded that "the rhythm of action currents appearing in the electromyogram of human voluntary contraction is no direct index of the rhythm of central innervation involved in the act." On account of evaluations of the absolute and relative refractory periods of nerve and muscle, as known for cold-blooded animals from the work of Keith Lucas and Adrian, Forbes and Rappleye came to the view that the spinal motor mechanisms discharge impulses at a frequency of 300-400, perhaps up even to 1000 per second. With this high frequency, at least with that of 1000 per second, many impulses will fall within the absolute or the beginning of the relative refractory period, so that according to these authors the muscle will respond with a frequency of 100-150 per second only. They based their reasoning on some experimental data of Beritoff, who showed that the muscle of the frog during reflex stimulation exactly follows the rhythm of the afferent stimulation up to a frequency of 250 per second, but that at higher frequencies of stimulation the frequency of the action currents went down to 150 per second, and in some parts of the curves down to 100 per second. The possibility of the hypothesis of Forbes and Rappleye must therefore be admitted. Later investigations of Cooper and Adrian(10), however, lead to the conclusion that it is very improbable that the frequency of the spinal centrifugal impulses during central innervation is too high to be followed by the muscle.

In support of their view, that the frequency of the muscular action currents is not the expression of the central nervous impulses, Forbes and Rappleye argued as follows(9, p 243) "The change of rhythm attending change of temperature would be inexplicable on any such basis. For why should a change in the temperature of the muscle cause a change in the frequency of discharge of impulses from the ganglion cells, whose temperature remains constant? This might conceivably be, if afferent impulses coming from the muscle modified the nerve centres so as to alter its frequency of discharge, but this assumption is far fetched and invokes nervous influences to which we know of no analogy." With regard to this we may remark that such a state of things is quite possible. Beritoff, in 1913, argued that the frequency of the reflex response will

be determined by that part of the reflex arc which has the longest refractory period, the experiments of Cooper and Adrian have shown that striped frog muscle when cooled reacts so slowly that it becomes the limiting factor. It is probable that the warm-blooded muscle will be still more sensitive in this respect. Furthermore, we have at present various experimental data which show that the "assumption," which Forbes and Rappleye in 1917 could call "far fetched," must in fact be made, for we know that various afferent impulses play an important rôle in the genesis of the high frequency of the action currents under central innervation.

Some years ago one of us (4) showed that the frequency of the muscular action currents in decerebrate rigidity and during voluntary contraction of human muscle is distinctly diminished after abolition of the proprioceptive impulses from the contracting muscle. For the human muscle he abolished them by intramuscular injection of novocaine, in the cat by section of the posterior roots. But it is not only proprioceptive impulses which have an influence in this respect, for the same author also showed that impulses from the labyrinths have, in many cases, an analogous influence. Further, one of us, together with J. B. Zwaardemaker (11), found that after section of the vagi in the neck, or blocking of these nerves by local novocaine poisoning, the frequency of the action currents of the diaphragm during its respiratory contractions undergoes a distinct change, in this case an augmentation.

The experimental data mentioned above show that various afferent impulses have an important influence on the frequency of the action currents of a contracting muscle, this factor may, therefore, have played a rôle in the cooling experiments of Buchanan, Forbes and Rappleye.

From their experiments on frog muscle Cooper and Adrian came to the conclusion that the frequency of the spinal impulses is the same as that of the action currents in the electromyogram. We might mention also the experiments of Athanasiu (12), from which he concludes that the nervous impulses going to a mammalian muscle have a frequency of 300-550 per second, but the views of this investigator have been fully criticised by Lapicque (13), Cooper and Adrian, and Forbes and McKee Cattell (14), we think therefore that we need not dwell upon them here.

The above-mentioned experimental data by Dusser de Barenne were the starting-point for the researches related in this paper.

The frequency of the action currents of a muscle during contractions of central origin, when all, or nearly all, afferent muscles are abolished

We thought that this problem might be attacked in the following way. A portion of the central nervous system, *e g* the cervical part of the spinal cord, is separated by transection at the medulla oblongata and at the junction of the cervical and thoracic spinal cord. The dorsal surface of the cervical cord is exposed. By touching this surface contractions of an arm muscle, *e g* the triceps brachii, can be elicited and its action currents recorded with a string galvanometer. Then all the posterior roots of the isolated cervical cord are cut and again by mechanical stimulation of the cord at the same spot a contraction of the triceps evoked and its action currents taken. Finally, the sensory mechanisms in the posterior grey matter of the cervical cord are narcotised by local application of novocaine. Again the muscle is made to contract in the same way. What is the frequency of its action currents in these several conditions?

Method Thirty-eight experiments were made on the cat and a few on the frog. The experiments on the cat were made on the decapitate preparation after Sherrington, with the modification that after the transection of the medulla at the calamus scriptorius, the head of the animal was not cut off but kept fixed in the mouth-holder as a good fixing-point. The temperature of the animal was kept at 38° C with a contact-thermometer in the rectum and electric lamps under the operation table.

Before transection of the medulla the dorsal surface of the spinal cord was exposed from the 1st cervical down to the 3rd or 4th thoracic, special care being taken to injure as little as possible the segmental blood supply. Immediately after transection at the calamus scriptorius the ether narcosis was discontinued and artificial respiration begun.

After exposure of the dorsal surface of the cervical cord, the electrodes, by which the action currents were led off to the galvanometer, were thrust into the muscle, we made use of silver needle-electrodes, coated galvanoplastically with a layer of AgCl. For the application of these electrodes a small hole was made in the skin, leaving the subcutaneous fascia over the muscle intact. All experiments in which the cervical cord was isolated and exposed were made on the triceps brachii. Some experiments were made on the M gastrocnemius, in these the lumbosacral region was exposed and a second transection made of one of the last thoracic segments. The muscle, the action currents of which were registered, was stretched so that the contraction of the muscle was

practically isometric, this has the great advantage that nearly no displacement of the string through "deformation currents" occurs

The use of needle-electrodes (Rehn), especially of Ag—AgCl electrodes, in this kind of work is of great advantage, because (1) the great electric resistance of the skin is eliminated, (2) it is possible to lead off from a very small number of muscle fibres. The Ag—AgCl electrodes, although not absolutely non polarisable, are practically non polarisable as a matter of fact the polarity of the electrodes in experiments such as these matters very little, if at all, because of the very rapid, very weak and alternating character of the action currents

The electrodes were always thrust into the distal third of the muscle and in the direction of its long axis, the electrodes then lie both on the same side of the "nervous equator" of the muscle and as nearly as possible parallel to the muscle fibres. The distance of the electrodes from each other was always $\frac{3}{4}$ to 1 cm and their depth in the muscle substance $\frac{1}{2}$ to $\frac{3}{4}$ cm. The operation table was electrically insulated by putting it on large paraffin blocks

The type of string galvanometer used was the large model of Edelmann, the current through its electromagnets being 3–3.5 amperes. Its string was a gold string of 2μ diameter, the tension of which was always adjusted so that it was nearly aperiodic in its deflection, and with this tension quite regularly followed vibrations up to 660 or 670 per second

Contraction of the muscle was produced, as described above, by gently touching the dorsal surface of the spinal cord, *i.e.* for contraction of the triceps brachii the dorsal surface of the 6th cervical segment, once or twice a second, in this way it is easy to obtain fair "tetanic" contractions of the muscle although the external stimulus is practically not periodic. Between these mechanical stimulations of the spinal cord, the exposed part of it was covered with a moist piece of cotton wool and this again with a large dry pad of cotton wool. In this way local temperature changes of the cord were, as far as possible, avoided

After the registration of the normal tetanus of the muscle, the dorsal spinal roots of the exposed portion of the cord were, in the earlier experiments, cut on both sides, *i.e.* when experimenting on the cervical cord, all the posterior roots from C 1 down to Th 3 or 4 were cut. Then a contraction of the muscle by mechanical stimulation of the cord was again elicited and its action currents taken. In the later experiments, when it was found that the frequency of the action currents showed no decrease through this de-afferentation of the muscle, this operative procedure was omitted. After the registration of the normal electromyogram the next step was made directly, *i.e.* the opening of the dura

for the local application of the novocaine solution (of 1 or 2 p c) on the whole dorsal surface of the exposed portion of the cord. Then every 5 or 10 minutes a contraction of the muscle was elicited in the way described above and its action currents registered. The "time" was recorded in all curves by a chronoscope of 10 double vibrations per second. Because of the irregularity of the action currents in these experiments, just as in our former researches on action currents under central innervation, we counted *all* the waves in the record, however small, over as large an area of the curve as possible, nearly always over 0.5 second, sometimes even over 1 second, exceptionally over 0.3 second.

Experimental results It was soon found that the activity of the isolated portion of the spinal cord, after transection at the 3rd-4th thor was in general greatly depressed and that in some cases it disappeared. This diminution is readily explained by the profound depression of the arterial blood-pressure, which occurs after this transection. In some preparations, in which the excitability of the isolated spinal cord remained good, notwithstanding this factor, it was found that the frequency of the action currents before and after transection at the level of the 3rd or 4th thor was the same, therefore, we have in the later experiments omitted this transection and worked on the decapitate preparation, in which the functional activity of the cord remains good for hours.

We first studied the influence of blocking of all afferent impulses from the periphery by section of the posterior roots of the isolated part of the spinal cord. We found that in nearly all experiments no, or no appreciable, diminution of the frequency of the action currents of the contracting muscle after section of the posterior roots could be observed.

The subjoining protocol and Figs 1 and 2 show this distinctly.

Exp 1 Cat. Ether narcosis, tracheal cannula, artificial heating, carotids tied, vagi cut, artificial respiration, exposure of spinal cord from C 1 to Th 4. 3.30 Decapitation. Ether off. Ag—AgCl electrodes in right M. triceps. 3.48 Mechanical stimulation of the dorsal surface of C 6 by touching once or twice a second. rectal temperature 37.8° C. Frequency of the action currents of the contracting M. triceps $97 \times 2 = 194$ per second (see Fig 1). 3.53–3.58 Section of posterior roots on both sides from C 2 to Th 3 (controlled by autopsy). 4.02 Mechanical stimulation as before, rectal temperature 37.9° C. Frequency of action currents of M. triceps 96 to $97 \times 2 = 193$ per second (see Fig 2).

From this and similar experiments, it may be concluded that the frequency of the action currents is not diminished by section of the series of posterior roots, even a few minutes only after the de-afferentation. It might seem that this fact is in contradiction with the experiments

of one of us (4), already mentioned. There is, however, a very plausible explanation for this discrepancy. In these experiments the proprioceptive impulses from the contracting muscle were of course cut off, but



Fig 1

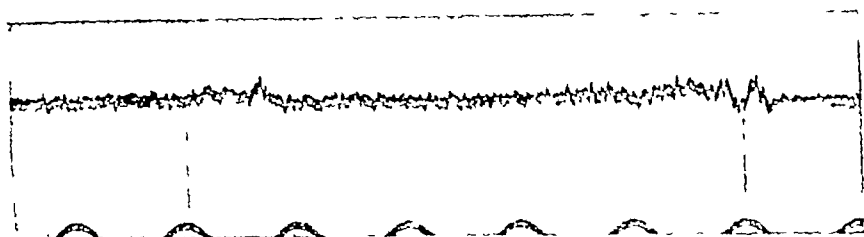


Fig 2

by the mechanical stimulation of the spinal cord its sensory mechanisms in the posterior horns are stimulated directly and thereby the peripheral proprioceptive impulses are replaced by artificial central impulses.

Because the de-afferentation had no effect on the frequency of the action currents under the conditions of these experiments, we have in our later experiments omitted also this operation, which is of course of great advantage for the functional condition of the spinal cord, since its blood supply is less interfered with. Therefore, in the later experiments, after recording the normal electromyogram, we immediately proceeded to the local narcotisation of the sensory spinal mechanisms by the application of the novocaine solution.

The result of these experiments and also of those where we applied the novocaine after section of the posterior roots has, without exception, been the following. In all experiments there was a distinct diminution of the frequency of the action currents after the local narcosis of the sensory spinal mechanisms. This in many experiments increased gradually with progress of the narcosis. In some experiments, even, a very slow, very regular rhythm developed.

It is perhaps best to consider here what we believe to occur in these experiments during the mechanical stimulation of the spinal cord. By touching the dorsal surface of the spinal cord, probably not only the intraspinal posterior root fibres are stimulated, but also the nerve cells of the posterior horns (the "Schaltneurone") and the motor neurones in the ventral gray matter. The excitations elicited in all these systems finally flow out along the motor nerve fibres and give rise to the "tetanic" contraction of the muscle with its concomitant electrical phenomena. By the local novocaine application the two first-named systems will gradually become more and more narcotised, so that with progressive local narcosis only the motor neurones, shielded by their ventral position, can transmit their excitation to the motor nerve fibres.

It is of course impossible to pick out in every experiment a definite stage of local narcosis, so that more or less accidentally, in some experiments we were able to observe a fairly gradual diminution of the frequency of the action currents, whereas in others there was quick disappearance of all action currents. In some of the experiments there was a stage in which the action currents became not only less frequent and more regular, but in which a very regular rhythm of the action currents, a true "sinusoidal" rhythm, developed. The subjoining protocols and figures will substantiate the above.

Exp 2 Cat Preparation as in *Exp 1* Spinal cord exposed from C 1 to Th 3 11 25 Decapitation Ether off Ag—AgCl electrodes in right triceps 1 50 Slight mechanical stimulation of dorsal surface of cervical segment 6 once or twice a second Action currents of M triceps recorded. Frequency 100 to $101 \times 2 = 201$ per second (see Fig 3) rectal temp 37.2°C 2 05–2 10 Posterior roots from C 1 to Th. 3 on both sides



Fig 3

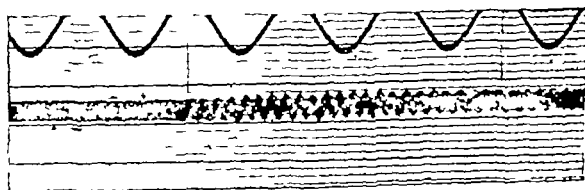


Fig 4

cut 2 32 Local application of 1 p c novocaine solution on dorsal surface of spinal cord from C 1 to Th. 3 3 02 Mechanical stimulation of dorsal surface of C 6 as above

Frequency of action currents $23 \times 1\frac{1}{2} = \text{ca } 77$ per second (see Fig 4) Rectal temp 37.1°C

In these curves can be seen (1) a very distinct diminution of the frequency of 61.7 p.c. of the action currents of the muscle after the novocaine poisoning of the spinal cord, (2) the great regularity of the action currents after the local narcosis. Another experiment and set of curves are the following

Exp 3 Cat Preparation as in *Exp 1* Spinal cord exposed from upper limit C 1 to Th. 3 Nearly no bleeding 2.20 Decapitation Ag—AgCl electrodes in distal third part of right M. triceps, ca 1 cm deep and at 1 cm distance from each other Rectal temp is rather low in consequence of delay in heating 3.10 Mechanical stimulation of dorsal surface of C 6 as usual. Action currents are shown in Fig 5 Rectal temp 35°C Frequency $76 \times 2 = 152$ per second. 4.02 Opening of the dura and local application of a 2 p.c. novocaine on the dorsal surface of spinal cord from C 1 to Th 3 4.24 Mechanical stimulation of C 6 as before Action currents of muscle, see Fig 6 Rectal temp 34.7°C Frequency $29 \times 2 = 58$ per second The reflex preparation reacts very well until at 4.35 the preparation is destroyed

Fig 5



Fig 6



The frequency of the action currents of the normal preparation is in this experiment somewhat lower than usual, probably because of the low rectal temperature, but still it is very clear that after the application of the novocaine a very large diminution of the frequency, in this case from 145 to 56 per second, i.e. a diminution of 89 out of 145, i.e. of 61 p.c. occurred. The great regularity of the action currents after the novocaine application is also very striking.

One of the experiments in which a gradual diminution of the frequency of the action currents, as well as a gradual decline in amplitude and a gradual developing regularity of the action currents occurred, is the following

Exp 4. Cat Preparation as in *Exp 1* 2.25 Decapitation 3.01 Action currents

Fig 7



Fig 8



Fig 9

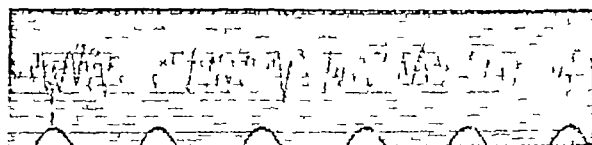


Fig 10

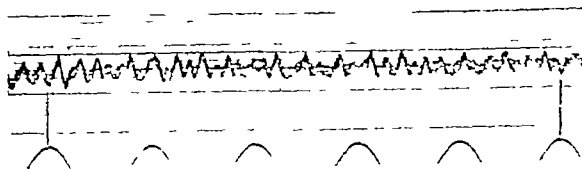


Fig 11

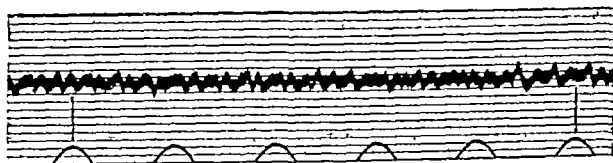
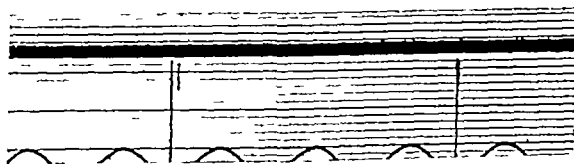


Fig 12



of Fig 7 Rectal temp 33.5°C Frequency $57 \times 2 = 114$ per second 3 02 Action currents of Fig 8 recorded this contraction of the muscle was an after discharge after the stimulation of Fig 7 Frequency 104 per second Since the frequency in Fig 8 was somewhat lower than in Fig 7, we waited a quarter of an hour to see if the frequency remained constant 3 15 Action currents of Fig 9 Frequency $53 \times 2 = 106$ per second 3 23 Application of 2 p c novocaine to the dorsal surface of spinal cord from C 1 to Th. 3 30 Action currents of Fig 10 recorded. Rectal temp 36°C Frequency $48 \times 2 = 96$ per second. 3 39 Frequency of action currents as in Fig 11 i e $42 \times 2 = 84$ per second. Rectal temp 36°C 4 05 Action currents of Fig 12 Rectal temp 36°C Frequency $16 \times \frac{1}{2} = 53$ per second

In this experiment also the diminution in frequency is prominent, being 53 p c The gradual development of the final very regular type of action currents is also very clear

As we have said, all our experiments on the cat yielded the same result, but we thought it advisable to make also some experiments on the frog The following is an example

Fig 13

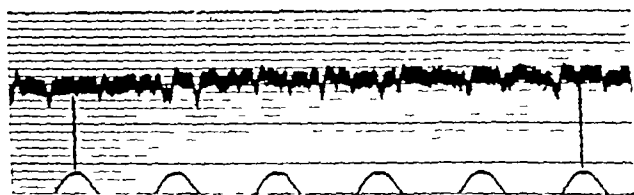
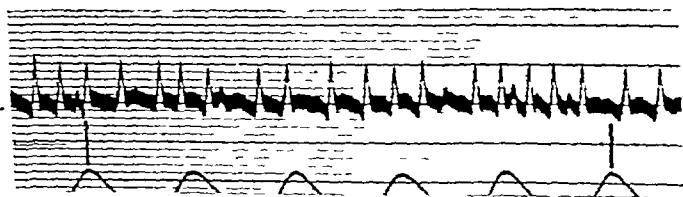


Fig 14.



Exp 5 *Rana* etc. 9 30 Transection of the spinal cord directly behind the occiput Brain destroyed. Exposure of spinal cord over its full length. After that prompt reflexes Cord covered with wet cotton wool and animal left to itself until the afternoon (room temp 8°C) 1 30 Animal pinned on a paraffin block in prone position, hind legs stretched and in maximal abduction. The joints of the legs are also pinned to the block and the pelvis fixed. Ag—AgCl electrodes in left M. semimembranosus at about 5 mm distance from each other 1 50 Prompt reflexes on pinching of toes and other parts of the body 2 30 The spinal cord in its caudal region is touched once very slightly with the end of a piece of cotton wool, twisted round forceps Strong contraction of the muscle its action currents are given in Fig 13 Frequency $51 \times 2 = 102$ per second. 2 44. Local application of 2 p c novocaine in frog Ringer on dorsal surface of the whole spinal cord. 2 50 Prompt reflexes, though less strong than 1 30, on pinching Mechanical stimulation of spinal cord as above Frequency of action currents $40 \times 2 = 80$ per second (Fig 14) 2 55 No reflexes elicitable On mechanical stimulation of the spinal cord in the way described, a distinct, though feeble

contraction of the M semimembranosus is elicited, its action currents are given in Fig 15 Frequency 50 per second of a nearly perfect sinusoidal regularity

From this example it will be seen that in the frog the same result is obtained as in the cat and that a perfectly regular type of action currents develops In Fig 15 it will be seen that only in one place, at the arrow, the regularity of the curve is broken The rhythm is so regular that one might think of an artefact from stray alternating currents This, however, is not the case, without excitation the string was perfectly at rest

Discussion

Since in the records of our experiments the action currents after the local novocaine poisoning often become smaller, it might be thought that the reduction in size would lead to the disappearance of some of the minor elevations of the curves Even if we accept this explanation to some extent, it cannot be accepted on the whole We have shown records in which, notwithstanding a distinct diminution in amplitude of the action currents, their frequency remained unchanged and we have even given curves where the very slow and regular action currents of the final stages of the experiment are larger than most of the action currents of the corresponding normal electromyogram Thus in Figs 3 and 4 there is a large difference in size of most of the action currents, while the frequency is the same In Fig 4 the frequency is only 77 per second, in Fig 3, on the contrary, 201, although it is very clear that the amplitude of most of the action currents in this last figure is much smaller even than in Fig 4 with the low frequency

Another explanation of the diminution of frequency might be looked for in the fact that the contraction of the muscle becomes somewhat smaller during the progression of the local narcosis of the sensory spinal mechanisms We cannot

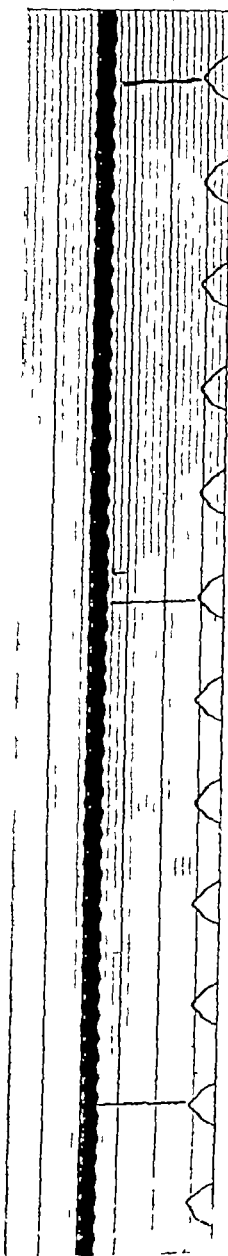


Fig 15

accept this explanation either. Various workers have shown already that, at least in warm-blooded animals, the frequency of the action currents of contractions of different strengths remains the same. Piper, Dittler, Forbes, Garten and Dusser de Barenne have shown this for various kinds of contraction under central innervation. In these experiments we confirmed this statement. Contractions of different strength in the same animal also gave the same frequency of action currents, even during the decline of a contraction the frequency is the same as during the acme of another contraction. Figs 16 and 17 show this distinctly, the frequency in both curves is the same, 140 per second, although Fig 16 is taken during the acme of a vigorous contraction, as shown by the large amplitude of the action currents, whereas Fig 17 was taken during a weaker contraction of the same muscle. That this contraction was less powerful, is readily shown by its smaller action currents.

Fig 16

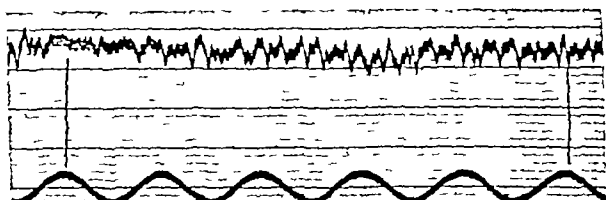


Fig 17



The same is also clearly shown in Fig 4, where we had the opportunity to take the action currents of a rather short contraction, so that we got on the record the action currents at the beginning, the acme and the decline of the contraction. At first without excitation the string is at rest, but then small action currents begin which gradually become larger and then again smaller. It is clear also from this record that in all three stages of the muscular contraction the frequency of the action currents is exactly the same.

From these experimental data we may conclude that the diminution of the frequency of the action currents in the conditions of these experiments is not caused by the difference in height of the contractions in the different stages of the experiments. So far as we can see, we can draw

only one conclusion, *ie* that the diminution is caused by a diminution of the frequency of the impulses, descending from the central nervous system to the muscle. The most plausible explanation seems to us to be that with progressive narcosis of the sensory spinal mechanisms the number of centripetal impulses playing on the motor neurones gradually diminishes and that, when the final regular, slow type of action currents is developed, the dorsal sensory spinal mechanisms are wholly or nearly wholly eliminated. We therefore interpret this slow "sinusoidal" rhythm of action currents as representing the impulses from the motor neurones, the intrinsic rhythm of the motor neurones, freed from the interference of the numerous centripetal impulses, which play normally on them and give rise to the high frequency of the normal electromyogram. In this way we have also a plausible explanation for the irregularity, with regard to amplitude and succession of its action currents.

We must dwell a moment longer on this point. At the base of our conception lies the view that the action currents of the normal electromyogram under central innervation are the true image of the nervous impulses, descending from the nervous system to the muscle along the motor nerve fibres. The adversaries of this view explain the irregularity of the action currents in the normal electromyogram by the supposition that not all the motor impulses reach the muscle in unison, but that the impulses in the nerve fibres are always more or less out of phase. This explanation seems *a priori* the most plausible, but on a closer examination there are various arguments which speak against it. First of all there are the results of these experiments, for it is difficult to see why the action currents would then become gradually more and more regular, so that finally a perfectly regular type occurs, notwithstanding that the electrodes remain throughout the experiment in exactly the same position. A further difficulty in that view is the fact that the electromyograms led off with the funnel electrodes, as formerly used, are exactly of the same type as those with the needle electrodes, although it is clear that with the former kind a much larger number of muscle fibres are led off, and this ought to demonstrate itself in a more complicated electromyogram. The same argument can be brought against Lapicque's view (13) who remarks that the different chronaxies of the thicker and thinner nerve fibres may come into account. If this argument were valid, it is very difficult to understand how the action currents of a tendon reflex, *eg* the knee reflex, can be a smooth diphasic action current (a fact first shown by Wertheim Salomonson), because then also differences

in phase ought to be shown by a few smaller notches or waves, superposed upon the large diphasic action current

One might argue that the knee reflex is not a reflex, but a 'peripheral' phenomenon as Gowers stated long ago. We cannot accept this argument because, apart from other considerations, we have often found in these experiments with mechanical spinal stimulation muscle twitches which were accompanied by a single diphasic action current. This shows conclusively that a contraction of 'central' origin can be accompanied by one single diphasic action current.

A strong argument for our view seems to be given also by the experiments of Bass and Trendelenburg⁽¹⁵⁾ in which they led off simultaneously the action currents from two parts of a contracting muscle with two string galvanometers. They found that the two curves showed a very close resemblance when the distance between the two pairs of electrodes did not exceed 5-6 cm. This fact obviously must be interpreted as showing that the motor neurones to the muscle fibres the action currents of which are led off act completely or nearly completely in unison and are not out of phase.

From these considerations and those given in the introductory remarks, we adhere to the view that the irregular action currents led off under suitable conditions from a muscle contracting as a result of impulses from the central nervous system, are the representation of the irregular nervous impulses which flow to the muscle along its motor neurones.

We conclude, then, that the action currents of striped muscle under different forms of central innervation, such as voluntary contraction in man, contraction of the diaphragm during respiration, decerebrate rigidity, mechanical stimulation of spinal cord and other forms of reflex-contraction, show a high frequency (from about 120 to 200 per second in various species and individuals) and are quite irregular. This type of action currents has its origin in the fact that during the contraction there are set up in the muscle many centripetal proprioceptive impulses, which play reflexly upon the motor neurones and superpose themselves upon the intrinsic impulses discharged by these neurones. But not only do the proprioceptive impulses play a rôle in this respect, but also centripetal impulses from other sources as was shown in former researches by one of us^(4, 11), i.e. from other muscles, the labyrinths, and interoceptive impulses.

By the method given in this paper, it is possible to abolish all these various centripetal impulses and to isolate, so to say, the action currents of the impulses of the motor neurones, and thus to bring forth their proper rhythm. This proper rhythm is of a rather slow and very regular

type (about 50 to 70 per second), sometimes the action currents even give a nearly "sinusoidal" curve

SUMMARY

1 Through mechanical stimulation, once or twice a second, of the dorsal surface of the spinal cord of the decapitate cat and spinal frog, "tetanic" contractions of striped muscle can be elicited, accompanied by frequent and irregular action currents

2 The frequency of these action currents varies in different individuals from 100-200 per second 160 may be regarded as the average

3 These action currents resemble closely the action currents led off from striped muscle under other forms of central innervation, such as voluntary contraction in man, the respiratory contractions of the diaphragm and decerebrate rigidity

4 In the same individual the frequency of the action currents under central innervation is the same for different strengths of contraction What differs then is the amplitude of the action currents, this is larger in vigorous contractions, smaller in weaker contractions

5 After local narcosis of the sensory spinal mechanisms, by which the centripetal impulses upon the motor neurones are cut off, the frequency of the action currents is distinctly reduced With the gradual progression of this local narcosis the frequency of the action currents gradually becomes lower and lower, so that, finally, a frequency of only 50 to 70 action currents per second is present

6 Hand-in-hand with this diminution of the frequency, the irregularity of the normal electromyogram gradually disappears, until, finally, a very regular type of action currents, often a true "sinusoidal" rhythm occurs

7 These slow and regular action currents represent the "proper" rhythm of the impulses of the motor spinal mechanisms, of the motor neurones, free from the various centripetal impulses, which play normally upon the neurones and give rise to a change of the slow, regular proper rhythm into the frequent and quite irregular excitations shown by the normal electromyogram

8 The electromyogram of striped muscle during contractions of central origin is a true image of the central impulses, reaching the muscle from the central nervous system along the peripheral motor nerve fibres

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ON THE FACTORS CONCERNED IN THE PRODUCTION OF PULMONARY ŒDEMA

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IN most of the previous work on pulmonary œdema, stress has been laid on the part played by the mechanical factors involved in its production. In view of the ease with which the mechanical conditions of the circulation can be controlled in the heart-lung preparation, and the frequency with which at some time or other in the course of experiments employing this method œdema of the lungs supervenes, it seemed to us that a study of the factors influencing the onset of œdema in the heart-lung preparation might throw light on the general question of its causation. In this preparation it is easy to measure at any time the pressure in, and the flow through, any part of the circuit—whether in the heart cavities or the vessels—so that an analysis of the mechanical factors at work can be made at any given moment. Such control is impossible, as such measurements are impossible to procure, in the intact animal.

Methods The heart-lung preparation was prepared in the usual manner as described by Fuhner and Starling(1). Dogs were used in the experiments, anæsthetised by intravenous chloralose (0.1 gm per kilo) following ether-chloroform induction. The circulating fluid was defibrinated blood, a sufficient quantity being obtained by bleeding a second dog, and using the mixture. Systemic pressure was taken by a mercury manometer near the outflow from the heart, and pressures from a branch of the pulmonary artery to the upper lobe of the right lung, and from the right or left auricle were taken by water manometers. The coronary flow was measured by means of a Morawitz cannula introduced through the right auricle. The temperature was maintained at 36° C. The average weight of the dogs was 10 kilos.

The course of experimental pulmonary œdema is difficult to follow with any degree of accuracy. The indicators used have always been gross changes in the appearance of the lungs upon the collection of a sufficient amount of fluid, or the production of râles of various types. In order to

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obtain more exact knowledge as to the time of onset and progress of the œdema, we adopted a method suggested by Dr D T Harris, of determining the changes which occurred in the electrical conductivity of the lungs. As expected, this was found to increase up to a certain point, in proportion to the amount of œdema fluid which collected in the lungs. The late collection of fluid in the larger bronchi and trachea, however, did not appear to modify the conductivity to any appreciable extent.

The apparatus was the usual Wheatstone Bridge with an induction coil and telephone receiver, as used in determining the conductivity of electrolytes, current being supplied by two dry cells. (If it is so desired, audion valve amplification and a loud speaker can be added.)

The electrodes first used were two needles held fixed distances apart penetrating the lungs to fixed depths. Upon increasing the distance between the needles, however, it was found that a proportionate decrease in conductivity did not occur (Fig 1, A, B, C). In order to ascertain

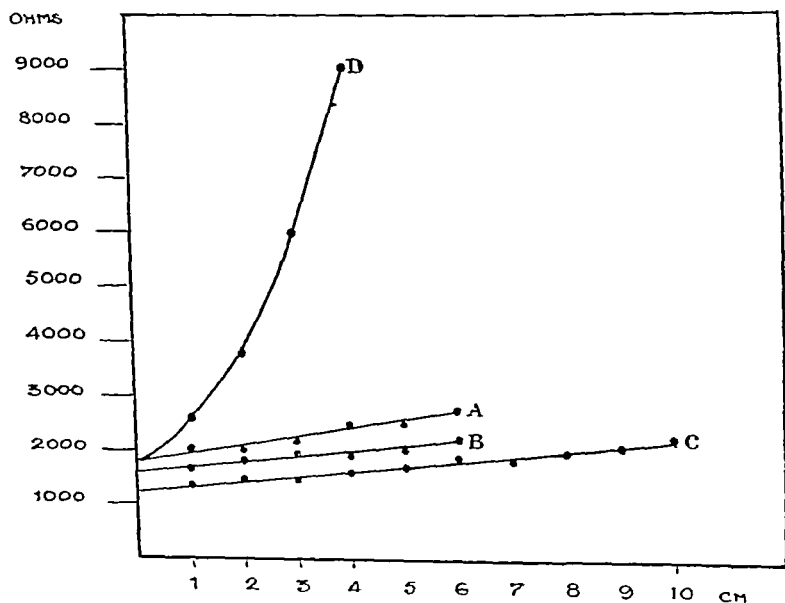


Fig 1 Measurements of conductivity along dead lung tissue with approximate curves A, B and C represent measurements along intact lung, D, along a strip of excised tissue. All the curves extrapolate to a finite quantity of ohms.

whether this was the case because of extremely large contact resistances, determinations were made upon excised strips of lung tissue, with small relatively uniform cross-sections of about 1 sq cm. On comparing the

curve thus obtained (Fig 1, *D*) with the former, the cause of the phenomenon became evident. The lines of current are apparently distributed throughout the lungs as in an electrolytic cell, the position of the contacts being of relatively little importance. As all these curves should extrapolate back to zero, the finite electrical resistance which appears by this means was interpreted as occurring at the contacts. Accordingly, although the initial amount of electrical resistance depends partially upon the electrodes, the changes which occur during the course of oedema were relatively the same whether these contacts consisted of two needles penetrating the lung, two plates applied to opposite sides of the lung, a needle imbedded in the tracheal wall with another penetrating the lung, or a needle in the tracheal wall with a plate applied to a lung.

The needle electrode on the tracheal wall, with a plate on the lung was finally adopted, as this method has several advantages over the others. Firstly, when a needle penetrates the lung, there is an initial increase in conductivity, presumably due to effusion around the injury. Secondly, the plate and needle involve but one variable contact, the tracheal electrode being kept constant by covering its entrance with a small wet pad. The variation in the other contact only occurs to any marked extent as the surface of the lung becomes wetter during the course of the oedema. And finally, the application of a suitably curved electrode (Fig 2) is exceedingly simple, the electrode being passed between

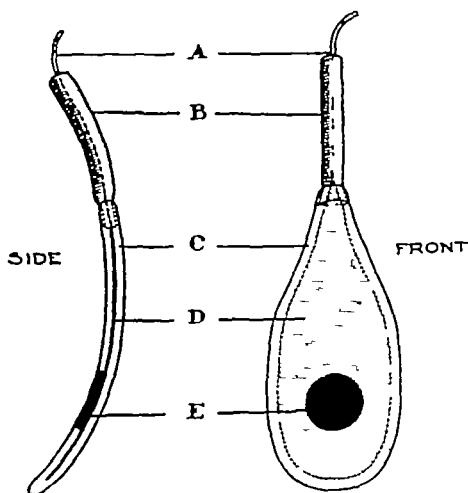


Fig 2 Plate electrode used on lung. *A*=wire, *B*=rubber insulation, *C*=paraffin shell, *D*=metal plate soldered to wire, *E*=exposed metal contact soldered to plate

the chest wall and the lung, and held *in situ* by the lung itself, which slips over it, but which does not alter the conductivity

Care must be taken to prevent certain sources of error. The trachea must not be allowed to dry around the needle electrode, the plate electrode must always be completely covered by the lung, and the degree of inflation of the lungs must be kept constant by controlling the apparatus used for artificial respiration. There is even a variation of the null point from inspiration to expiration, so that readings should be taken either at one phase or the other. There is little error caused by current short-circuiting around the other tissues of the animal. Measurements kindly made during an experiment by Mr L. Bayliss showed that a current of 5 micro-amperes passed along the chest wall parallel to one in the lungs of 30 micro-amperes. Furthermore, this fraction is a constant, and should not influence the cedema curve. On the other hand, there is considerable alteration in conductivity if a pool of blood be allowed to accumulate in the thoracic cavity, and this should be prevented by passing tube drains through the most dependent portions of the chest wall on either side.

The conductivity curve obtained during cedema was checked against the percentage weight of water gained by the lungs in the following manner. Pulmonary cedema was produced in a series of intact cats by intravenous infusion of normal saline solution. (It was not thought necessary to determine the effect of the added electrolyte to the blood, as precisely similar changes in conductivity were obtained when cedema was produced by several other less practical methods.) At intervals during the course of the infusion the conductivity was measured, and small portions of the lungs excised. These pieces were then dehydrated to constant weight in an oven at 110°C . It was found that a maximal gain in water of from 80 p.c. to 92 p.c. or 93 p.c. took place synchronously with the change in conductivity (Fig. 3). Variations in the blood content of the pieces of lung were apparently not sufficient to produce an error, as constant results were obtained, both for non-cedematous and fully cedematous specimens.

A further check was made to determine whether increased blood flow through the lungs would alter their conductivity. This was found not to be the case in the heart-lung preparation, where the amount of blood flowing through the lungs was varied by altering the inflow into the right heart.

In the present communication only those experiments will be considered in which pulmonary cedema developed spontaneously in the

absence of other complicating features, or in which it was artificially produced. In most instances the œdema came on slowly, but in others, generally as the result of some special procedure or accident, the onset was rapid. For the sake of convenience we have dealt with these two types of experiment separately, although probably no hard and fast line can be drawn between them.

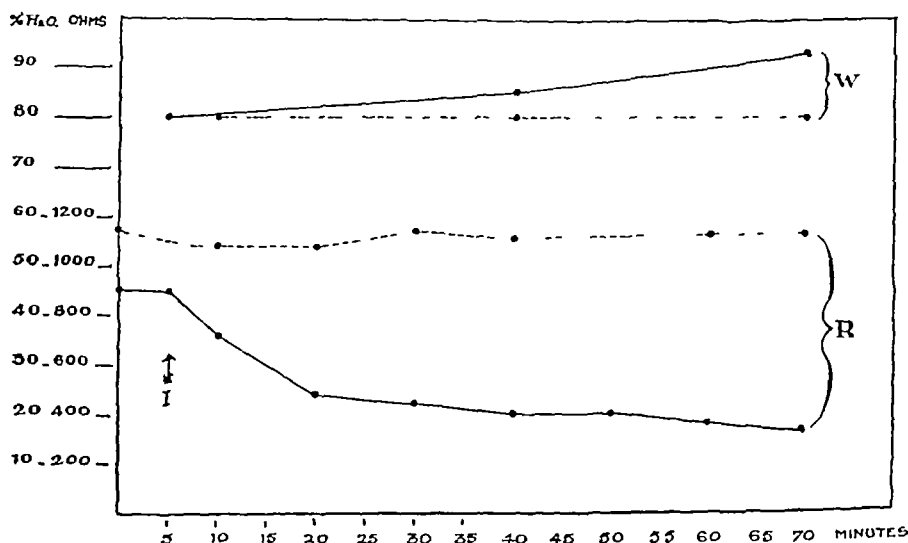


Fig 3 Measurements made on lungs of intact cats during pulmonary œdema caused by infusing normal saline. W = percentage of water by weight, R = resistance. Dotted lines signify controls.

Slow œdema

The heart-lung was prepared as rapidly as possible, the apparatus arranged for the observations, and then none but absolutely necessary manipulations performed until end-stage œdema was present. In eight experiments œdema appeared at any time from 1 to 4 hours after the completion of the preparation, the usual time being 3 hours.

In each of the experiments, the electrical resistance of the lungs dropped slowly, until when it had about halved its original amount (measured in ohms) the evidence of gross œdema was apparent. At this point the lungs were wet and moist râles were heard, but we shall refer to the stage as "early" œdema, for as yet no collection of fluid could be made out in the trachea. From this point on, very little change in conductivity took place, but within another $\frac{1}{2}$ to 1 hour, the larger bronchi

and trachea were filled with fluid. which stage we have called "late" œdema

From the beginning of the experiment to early œdema the coronary output gradually increased, the rise as a rule being absolutely synchronous with the change in conductivity, so that at the early œdema point the output per minute was usually about double its original figure. From early to late œdema, however, while the conductivity showed practically no alteration, there was a very striking increase in coronary flow so that some final readings were as high as five times the original figure.

Either the inflow to the heart or the output was kept constant during each experiment, usually the former. With a constant inflow, the output of the heart as measured in the usual manner diminished as more blood flowed through the coronaries. The total output (systemic plus coronary), however, kept fairly constant, in accordance with the recent work of Anrep and Bulatao⁽²⁾ slight variations occurring probably due to unavoidable changes of the inflow during measurement, etc.

Up to the time of early œdema there was uniformly a slight total rise in pulmonary arterial pressure of from 2-5 cm. water occasionally preceded by an original drop of from 2-3 cm. from the first reading (Fig 4). As removing the coronary cannula at early œdema gave a further rise up to 7 cm. proportionate with the amount of rise when the cannula was *in situ* most of the rise in the pulmonary arterial pressure of early œdema was probably due to the added inflow from the coronaries the initial rise when the cannula was *in situ* being from the venæ Thebesii and the posterior cardiac veins.

The pulmonary arterial pressure, in the interval between the onset of œdema and its later stages rose continuously and rapidly, and the final readings were usually at least triple those taken at the beginning of the experiment. As has been previously stated, the coronary output also increased markedly. At the end point it was not possible to obtain a further proportionate rise in pulmonary pressure by withdrawing the coronary cannula, and it is likely that other factors besides the increased inflow into the right heart contribute toward the causation of this extreme elevation of pressure. It seems likely to us that, in view of the obvious microscopic damage to the blood vessels, described later, changes in the lumina from desquamated and swollen intimal cells would be sufficient to cause a certain amount of damming back of the blood stream. At the same time the accumulation of fluid in the alveolar spaces may also exert sufficient pressure on the blood vessels to alter the resistance to passage of blood through them. Such suppositions of course cannot be

proved, but the facts are, that in this type of experiment the œdema and the increased coronary flow occur before the rise of pulmonary

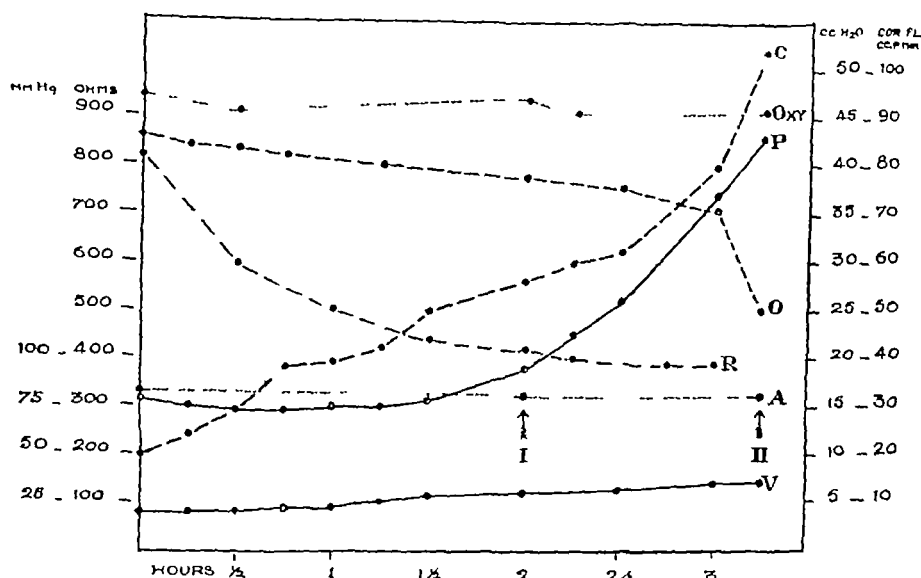


Fig 4 Spontaneous slow œdema. Oxygen given throughout. Inflow kept constant, and total output (systemic plus coronary) between 536 and 504 cc blood per minute. Early œdema present at I, and late œdema at II. C=coronary output in c.c. blood per minute, Oxy=percentage oxygen saturation of the blood, P=pulmonary arterial pressure in cm water, O=systemic output in c.c. blood per 10 seconds, R=electrical resistance of the lungs in ohms, A=systemic arterial pressure in mm mercury, V=inferior vena cava pressure in cm water

arterial pressure, and that in the early œdema the rise in coronary flow accounts for any rise in pulmonary arterial pressure which occurs

In no case was there more than a very slight increase in either auricular pressure up to the time of late œdema. In one experiment a sufficient degree of heart failure was present to produce a rise of right auricular pressure of 5 cm water. This was reduced to 3 cm by removing the pericardium (Starling(3)). At the same time, however, there was an increase in pulmonary arterial pressure of 23 cm water.

Desaturation of the blood was always present to a certain extent after the first hour or so of an experiment if no oxygen was given, and as œdema progressed it became very marked. Early œdema gave an average saturation of 74 p.c., while in late œdema readings of anywhere from 50 p.c. to 60 p.c. were obtained. In order to determine whether the increased coronary flow was a result of this condition of the blood

(Hilton and Eichholtz(4)), oxygen was given throughout two experiments. Even during the most marked œdema, sufficient oxygen passed into the remaining aerated alveoli to keep the saturation well up (Fig 4), and precisely the same changes in the coronary output, as well as in the other particulars of the experiment, took place.

As the rise in pulmonary pressure in these experiments was so evidently secondary, and as during the entire course of the œdema the coronary flow steadily increased, the initial changes were in all probability vascular. These changes were outwardly manifested by increased permeability and consequent œdema in the vessels of the lungs, and by dilatation in the vessels of the heart. The factors in the blood, or otherwise, upon which the vascular changes in this type of experiment depend, have not been determined, but will be discussed later.

Rapid œdema

In 4 experiments rapid pulmonary œdema was produced within $\frac{1}{2}$ to 1 hour, after the heart-lung had been prepared. As the cause of the

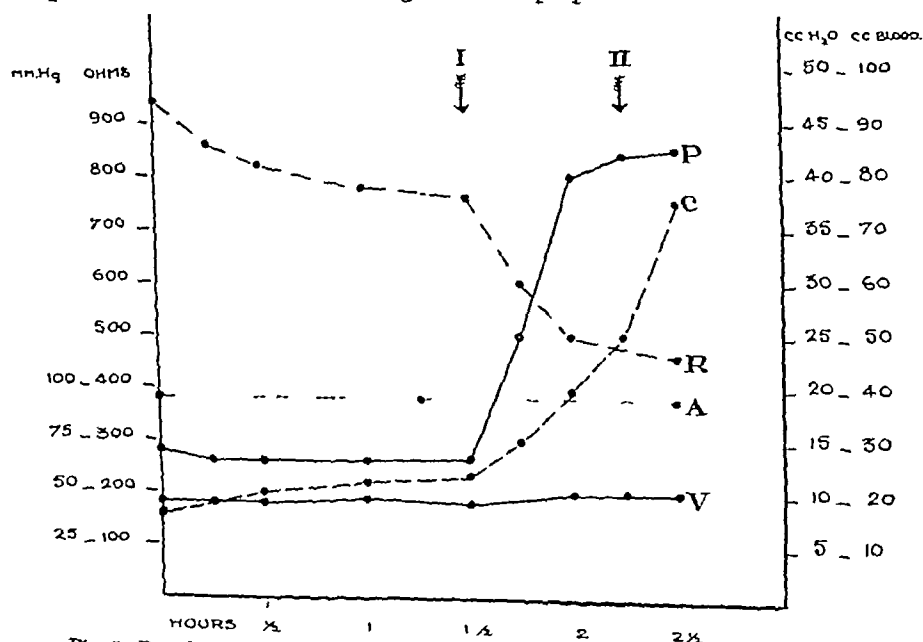


Fig 5 Rapid œdema caused by old blood. Oxygen not given. Inflow kept constant. No œdema present at I, 100 c.c. old blood added and 100 c.c. good blood withdrawn. Late œdema present at II. P=pulmonary arterial pressure in cm. water. C=coronary output in c.c. blood per minute. R=electrical resistance of the lungs in ohms, A=systemic arterial pressure in mm. mercury. V=inferior vena cava pressure in cm. water.

œdema was of the same general nature in two experiments, and probably as well in the third, and as all the observations were uniform, one typical experiment is shown in Fig 5

In the first experiment 100 c c of dog's blood, drawn the previous day and kept on ice, were added to the inflow reservoir and the same amount of good blood withdrawn. In the second, 5 c c of 1 p c silver nitrate solution was added to the reservoir. The third experiment was originally intended to demonstrate the usual form of slow œdema, but rapid œdema took place instead. The only reason that could be found was that ordinary ink had been used to colour the manometer solutions and that a certain amount had inadvertently made its way into the blood stream.

In these three experiments the early stages of œdema, so well marked in those previously described, were obliterated by the rapidity of progression from the onset to the final stage. In the first two experiments, the instillation of a toxic agent caused an immediate drop in the electrical resistance of the lung, and a rise of pulmonary arterial pressure to the level of late œdema. In the third experiment the pressure was high from the beginning and gradually rose to the end figure within a short time, somewhat confirming our belief as to the cause. The coronary flow, and all the other features of the experiments rapidly became identical with those in the late stage of slow œdema.

In one experiment, œdema was rapidly produced in an entirely different manner. A certain amount of torsion of the lobes of the right side was present at the beginning of the experiment, which caused a slight obstruction to the venous outflow of the lungs, while at the same time not impeding the arterial inflow. The pulmonary arterial pressure rose gradually to 52 cm water during the course of half an hour, and a well marked late stage œdema developed throughout the lungs. The auricular pressures remained low, and what is of extreme interest, the coronary flow only rose to 30 c c blood per minute, whereas in the other experiments it had often reached 90 to 100 c c at the end stages.

In the first three of these experiments the increased permeability of the lung vessels, and the dilatation of the cardiac vessels can be said to be due to a known toxic agent in the blood stream. The fourth experiment, in which œdema was produced by an entirely different mechanism, shows a relative absence of increased coronary outflow.

Histological changes in lungs and heart

In order to correlate, as far as possible, the data given above with the actual condition of the vessels, sections of the lungs and heart were prepared at various stages of œdema. The tissues were fixed in Zenker's solution, prepared in the usual manner, and stained either with hæmatoxylin-eosin, or hæmatoxylin-Bassini. Three sections are described.

1 *Lung of moderately advanced œdema* All the cells in the walls of arteries, arterioles and veins show swelling, while the nuclei of the intimal cells are occasionally poorly stained and broken. In some portions of the section there is even absence of the intimal cell nuclei. The capillaries are dilated and filled with erythrocytes, and their endothelial cells show similar nuclear changes. There is some swelling of the epithelial cells of the bronchial mucous membrane and occasional desquamation. There are collections of desquamated bronchial and alveolar cells in the lumina of the bronchi. The nuclei of the alveolar epithelial cells are generally normal in aspect, but the alveolar spaces are also filled with desquamated cells and amorphous masses, which probably consist of coagulated proteins.

2 *Lung of severe œdema* The intima of the arteries, arterioles and veins in this section, shows marked alterations in the appearance of the cell nuclei. These are frequently absent, and the remainder poorly stained and vacuolated. There is an escape of erythrocytes through the walls of the arterioles. The capillaries are dilated, partially filled, and their cells show similar nuclear changes. The bronchi and their branches, as well as the alveoli, show similar changes as in the previous section, to a more marked degree. The bronchial epithelial cells occasionally show vacuolisation as well as swelling.

3 *Heart of severe œdema* The intimal cells of the larger coronary branches have, as a rule, poorly stained nuclei, and there are aggregations of erythrocytes surrounding these vessels. The larger veins have the same appearance, but even to a more marked degree. The capillaries show exactly the same changes as in section 2. The heart muscle cells show some fragmentation, and in certain places are rolled up or wavy, but on the whole they appear normal, with well stained nuclei. Collections of erythrocytes are seen interspersed among the muscle cells.

More sections are not described as they all show essentially the same changes, the variations being of degree only. There is some difference in appearance at the various portions of each section, the above description representing the average picture. The principal pathological process seems to be one involving the intimal layers of the small vessels and the capillaries of the heart and lungs. There are also, however, degenerative changes of other structures to a lesser extent, evident from the appearance of alveolar and bronchial epithelium and the heart muscle.

In these sections we do not observe one of the changes commonly seen in human pulmonary œdema, namely the filling of alveolar spaces with coagulated œdema proteins. This is probably due to the fact that the pieces of lung were excised and fixed immediately upon the inception of the various stages of œdema. At this time fluid can easily flow out and be expressed by the contraction of the tissue, while con-

œdema was of the same general nature in two experiments, and probably as well in the third, and as all the observations were uniform, one typical experiment is shown in Fig 5

In the first experiment 100 c c of dog's blood, drawn the previous day and kept on ice, were added to the inflow reservoir and the same amount of good blood withdrawn. In the second, 5 c c of 1 p c silver nitrate solution was added to the reservoir. The third experiment was originally intended to demonstrate the usual form of slow œdema, but rapid œdema took place instead. The only reason that could be found was that ordinary ink had been used to colour the manometer solutions and that a certain amount had inadvertently made its way into the blood stream.

In these three experiments the early stages of œdema, so well marked in those previously described, were obliterated by the rapidity of progression from the onset to the final stage. In the first two experiments, the instillation of a toxic agent caused an immediate drop in the electrical resistance of the lung, and a rise of pulmonary arterial pressure to the level of late œdema. In the third experiment the pressure was high from the beginning and gradually rose to the end figure within a short time, somewhat confirming our belief as to the cause. The coronary flow, and all the other features of the experiments rapidly became identical with those in the late stage of slow œdema.

In one experiment, œdema was rapidly produced in an entirely different manner. A certain amount of torsion of the lobes of the right side was present at the beginning of the experiment, which caused a slight obstruction to the venous outflow of the lungs, while at the same time not impeding the arterial inflow. The pulmonary arterial pressure rose gradually to 52 cm water during the course of half an hour, and a well marked late stage œdema developed throughout the lungs. The auricular pressures remained low, and what is of extreme interest, the coronary flow only rose to 30 c c blood per minute, whereas in the other experiments it had often reached 90 to 100 c c at the end stages.

In the first three of these experiments the increased permeability of the lung vessels, and the dilatation of the cardiac vessels can be said to be due to a known toxic agent in the blood stream. The fourth experiment, in which œdema was produced by an entirely different mechanism, shows a relative absence of increased coronary outflow.

of Anrep and Bulatao(2) proves that this is not the result of "back-pressure," but of the increased total output of the heart due to added coronary flow. Accordingly there is no more reason to believe that this means alone could produce œdema, than directly altering the inflow.

In the case of true "back-pressure" œdema, the left ventricle is not able to express its contents completely, and the diastolic pressure is accordingly raised by the residual blood within it. At the same time dilatation takes place. Owing to the ensuing inability of each of the chambers of the heart to fully express its contents, as well as to possible valvular incompetence, the increased pressure is then transmitted to the lungs and right heart in turn. The simple fact is often overlooked that beyond a certain point this process is part and parcel of cardiac failure, and the extremely high pressures to which the lung vessels may thus be subjected by a failing heart may be sufficient to produce transudation. Matsuoka(6) says "in the production of obstructive œdema the output of the heart may be diminished to a minimum, and the pressures in the pulmonary artery, the inferior vena cava, and the right auricle may be increased to a maximum, all quite independently of the height of the arterial pressure and the amount of venous inflow." This description is used to illustrate œdema brought about through increased systemic resistance, but merely indicates heart failure taking place because of an impossible burden. True "back-pressure" œdema may also occur when there is an obstruction in the pulmonary veins. This is experimentally possible, but most infrequent as a natural patho-physiological process save where there is embolism or a left auricular thrombus (Wiggers(7)).

Another hypothetical mechanism of œdema, originally put forth by Welch in 1878, is a *dissociation of the outputs of the ventricles*. It is true that he produced pulmonary œdema in this manner by injuring the left ventricles of rabbits, but that such a condition can occur spontaneously has never been well established. Temporary differences in output take place upon alteration of the venous inflow directly, or through the medium of the peripheral resistance. But immediately upon the engorgement of the lung vessels, automatic regulation of the ventricles takes place, so that the condition becomes that which we have described before. Definite failure of the left ventricle is hardly conceivable, save for very short periods of time, without a concomitant failure on the right. Increased left intra-ventricular diastolic pressure is immediately transmitted to the right side, causing equal dilatation and failure, while any disturbance in coronary circulation instantly affects the nutrition of the musculature of the right heart. As any dissociation of output can only

siderable dehydration can take place during the preparation of the section

Possibly the most important question to be considered is what part of the apparent damage to the vessels is caused by some direct toxic action of unknown nature, and what part is caused by imbibition and passage of fluid through the cells during the course of the oedema. The only direct evidence we have to throw light on this point is that marked oedema caused by saline infusions shows but little vascular change when compared to the other varieties. In some respects, however, they are not quite comparable. But the simultaneous alterations in the microscopic picture of the heart is suggestive of the fact that the vessel changes are primary and not secondary to the oedema. We must reiterate, that despite the changes in the appearance of the heart muscle, no gross heart failure was present at any time.

Discussion

Most experimental work on pulmonary oedema has been by way of a search for a single etiological factor, to be applied under all circumstances. There are, however, a number of known factors, and presumably as many more unknown, which always participate. Their relative importance probably varies from case to case. We will deal here, in the light of our experiments, with the different factors to which the preponderant part in the production of lung oedema has been ascribed by various writers on the subject.

Increased pressure in the pulmonary blood vessels When the heart and lungs are normal, the chief cause for the elevation of pulmonary arterial pressure is an increased venous inflow to the heart. As was shown by Patterson, Piper and Starling⁽⁵⁾ in the heart-lung preparation, the circulation rate under these circumstances is increased to a marked degree through cardiac adaptation, without any other signal disturbance, and without altering the pulse rate. Moreover, in normal animals, the added compensatory mechanism of an increased pulse rate takes part. In the absence of cardiac failure, with the circulation intact, there is no reason to believe that by this means a sufficiently great filtration pressure is produced in the pulmonary vessels to cause transudation. Of course, such a rise of pulmonary pressure as is incidental to the generally increased circulation rate may accelerate the production of oedema in the presence of any other factors.

As was described by Fuhner and Starling⁽¹⁾, increasing the peripheral resistance will also increase the pulmonary pressure. The work

of Anrep and Bulatao(2) proves that this is not the result of "back-pressure," but of the increased total output of the heart due to added coronary flow. Accordingly there is no more reason to believe that this means alone could produce oedema, than directly altering the inflow.

In the case of true "back-pressure" oedema, the left ventricle is not able to express its contents completely, and the diastolic pressure is accordingly raised by the residual blood within it. At the same time dilatation takes place. Owing to the ensuing inability of each of the chambers of the heart to fully express its contents, as well as to possible valvular incompetence, the increased pressure is then transmitted to the lungs and right heart in turn. The simple fact is often overlooked that beyond a certain point this process is part and parcel of cardiac failure, and the extremely high pressures to which the lung vessels may thus be subjected by a failing heart may be sufficient to produce transudation. Matsuoka(6) says "in the production of obstructive oedema the output of the heart may be diminished to a minimum, and the pressures in the pulmonary artery, the inferior vena cava, and the right auricle may be increased to a maximum, all quite independently of the height of the arterial pressure and the amount of venous inflow." This description is used to illustrate oedema brought about through increased systemic resistance, but merely indicates heart failure taking place because of an impossible burden. True "back-pressure" oedema may also occur when there is an obstruction in the pulmonary veins. This is experimentally possible, but most infrequent as a natural patho-physiological process save where there is embolism or a left auricular thrombus (Wiggers(7)).

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occur as an extremely transitory phenomenon, we do not believe that *per se* it can play any important part in the causation of pulmonary oedema

In our experiments it has become evident to us what heights the pulmonary pressure may reach, in the absence of cardiac failure, secondary to oedema of the lungs. When this condition obtains, however, heart failure is imminent, in which circumstances a vicious circle would be established. Here lies the value of decreasing the venous inflow to the heart, *i e* clinically by venesection, and preventing such a condition from occurring, by lowering the already high pulmonary pressure.

Changes in the blood Changes in the blood may produce oedema, as can readily be demonstrated, in fact Barry(8) believes the heart-lung oedema to be caused mainly by dilution of the blood colloids. Saline infusions probably act in this manner, it being difficult to believe that small amounts of normal saline added to the blood should act other than by facilitating simple filtration. Such substances which when added to the blood, or produced therein, will deleteriously affect the blood vessels, are discussed below.

Changes in the blood vessels It must be remembered that in the heart-lung preparation, at the present time, it is necessary to use defibrinated blood as the circulating medium. Such blood has undergone profound changes in the process of defibrination, which, without interfering with its functions as a carrier of oxygen and carbon dioxide, as well as of soluble food and waste products, have given rise to the production of undefined substances which may have a toxic effect on the blood vessels and tissues. The strong vasotonic action of such blood is well known, and Eichholtz and Verney(9) have shown that this action makes it impossible to maintain circulation through an excised kidney by means of a mechanical pump. The circulation in a heart-lung kidney preparation is possible only because the lungs and possibly the heart exert some kind of detoxicating influence on the blood, removing its constricting effect on the renal vessels. A few minutes standing in glass leads to the fresh formation of the toxins, so that after 24 hours, kept defibrinated blood becomes extremely toxic for the heart-lung preparation.

But the absorption of these toxins in the heart-lung preparation is not without deleterious effects. Microscopic examination shows gradually increasing defects in the intima of the blood vessels and in the capillary endothelium. Any such change is known to increase the permeability of the capillaries—and we are therefore justified in regarding these capillary changes as the prime factor in the causation of the pulmonary

œdema Any mechanical factors are only secondary in importance If, however, the toxic action of the blood is excessive, as is the case when 24 hours old defibrinated blood is used, or when salts of the heavy metals are added, a marked change is produced at once in the capillary endothelium, giving rise to increased resistance and stasis in the capillaries and a marked rise of pulmonary arterial pressure But this rise of pressure is secondary to the endothelial change, which is responsible for the increased exudation and œdema, and cannot itself be regarded as the cause of the œdema

The great rise of pulmonary arterial pressure which comes on at the end of an experiment, when massive œdema of the lungs has already developed, is probably due to the changes in the vessel walls and the pressure exerted by the fluid filling the intercapillary spaces

We should be inclined to ascribe a similar pathogenesis to many cases of acute pulmonary œdema in man, and to regard a toxic influence on the capillary walls as primarily responsible for the increased exudation In these cases the extreme secondary rise in pulmonary pressure is probably also present Furthermore, as not all clinical cardiac failure is accompanied by pulmonary œdema, the question arises as to whether in cardiac œdema some direct action of the blood on the capillaries does not take place either through anoxæmia or the formation of toxic substances

CONCLUSIONS

1 A method is described for following the course of pulmonary œdema

2 In the heart-lung preparation, lung œdema supervenes at varying periods after the beginning of the experiment In most cases the onset is gradual and a number of hours elapse before the œdema becomes so marked as to put an end to the experiment Occasionally the œdema is much more rapid in onset, and it is always produced within a very short time if defibrinated blood which has stood for 24 hours, or metallic poisons are added to the circulating fluid.

3 In both cases, namely slow and rapid œdema, degenerative changes are found in the intimal cells of the blood vessels and in the capillary endothelium of the lungs and heart

4 The principal cause of the œdema is the injury to the capillary endothelium, and the greater permeability thereby produced

5 There is a gradual steady increase in the flow through the coronary vessels in the course of the experiment—which may amount to five times the original rate Since changes in the intima of the cardiac vessels are

observed similar to those described in the lungs, we regard this increase as the response of the coronary vessels to the toxic effect exerted by the defibrinated blood

6 The œdema, in its initial stages, is unattended by any changes in the resistance to the flow of blood through the lungs, as judged by the pulmonary arterial pressure. The small rise of pulmonary pressure observed is due entirely to the greater flow through the coronary vessels.

7 In the late stages of both slow and rapid œdema, there is a secondary marked rise in pulmonary arterial pressure. This is probably due to the mechanical influence of the œdema fluid on the pulmonary blood vessels, and to changes in the blood vessel walls themselves.

8 We suggest that in certain cases of pulmonary œdema, as observed clinically, similar effects may take place through toxic action of the blood on the pulmonary vessels. This factor may enter into the causation of cardiac œdema.

This work was conducted in the laboratory of Prof. E. H. Starling, and we are greatly indebted to him for his continued guidance and help. We also wish to express our thanks to Prof. A. V. Hill and Dr. Anrep for many valuable suggestions.

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THE *IN VIVO* PERMEABILITY OF THE RED CORPUSCLES OF THE RABBIT BY J T IRVING
(Benn W Levy Student, Frank Smart Student, Gonville and
Cairns College) AND H D KAY (Beit Memorial Fellow)

(From the Biochemical Laboratory, Cambridge)

THE experiments recorded in this paper were carried out in an endeavour to obtain evidence for or against the hypothesis adumbrated by Haldane, Kay and Smith⁽¹⁾ that the administration of insulin to the rabbit brings about permeability changes in certain of its body cells

In two preliminary experiments the partition of "sugar" or, better, reducing substance between the corpuscles and the plasma was determined by Hagedorn and Jensen's⁽²⁾ method before and at intervals after the injection of insulin, and figures were obtained which appeared to indicate that the reducing substance in the corpuscles did not decrease in a manner parallel with the decrease in plasma "sugar" during the hypoglycæmic period. It was decided to ascertain, if possible, whether any changes in the permeability of the red cells for reducing substance could be shown to be brought about by insulin injected *in vivo*.

In the early stages it was found that the partition of reducing substance between plasma and corpuscles was influenced very markedly (at least in the case of rabbit's blood) by the presence of anticoagulants, and that the addition of anticoagulants to blood *in vitro* completely obscured the actual state of affairs in the blood *in vivo*. The effect of anticoagulants may account for the fact that physiological literature is full of contradictory statements as to the distribution of "sugar," or even more hopefully "glucose," between the corpuscles and the plasma in the blood of a large number of animals. In the case of man some writers, e.g. Falta and Richtner-Quittner⁽³⁾, find no sugar¹ in the corpuscles, whilst Wu⁽⁴⁾ finds equal and Högler and Ueberrach⁽⁵⁾ nearly equal distribution. Most workers have found less sugar in rabbit's corpuscles than in rabbit's plasma, and a few recent workers (Ege⁽⁶⁾, van Crefeld and Brinkman⁽⁶⁾) state that there is no reducing substance in rabbit's corpuscles. We decided, before proceeding further, to

¹ Throughout this paper, wherever the word sugar is used, it should be regarded as between inverted commas

re-examine the distribution of reducing substance between the corpuscles and plasma under conditions as near as possible to those existing in the blood of a normal rabbit

Experiments using anticoagulants Blood was taken from the ear vein of large healthy rabbits (weight 3 to 4 kilos) direct into a little above the minimal quantity of anticoagulant in a small graduated centrifuge tube. Duplicate samples of blood were at once pipetted out for total blood sugar estimations, Hagedorn and Jensen's method being usually employed. In a few cases Calvert's(7) method was used, and gave similar figures, but was not found to be quite as reliable as the former method. The rest of the blood was immediately centrifuged, and duplicate samples of plasma for plasma sugar taken within 5 minutes of the drawing of the blood from the animal. The corpuscular sugar was calculated from these two figures and the hæmatocrit reading. In order to avoid the change in relative volume of the corpuscles which is brought about by the addition of anticoagulants of high osmotic pressure it was found necessary to take a small sample of the blood at the same time into a tube containing a trace of heparin, which, at the concentrations required to prevent clotting, has no appreciable osmotic pressure.

The anticoagulants we used were sodium citrate, 2.5 p.c., sodium fluoride, 1 p.c., potassium oxalate, 0.2 p.c., heparin, 0.02 p.c. In Table I are given a few figures illustrating the results of a large number of experiments. In the same table figures obtained from blood centrifuged in ice-cold paraffined tubes are included.

TABLE I (Reducing substance calculated as glucose)

Blood sugar	Plasma sugar	Hæmatocrit	Corpuscle sugar	Anticoagulant
124	136	28	093	Oxalate
127	139	33	101	Oxalate
111	124	32	084	Fluoride
111	119	32	097	Citrate
111	131	32	080	Heparin
108	132	37	068	Heparin
101	120	37	068	Citrate
108	129	37	073	Oxalate
Paraffined tubes				
129	170	33	045	Nil

These results show that using anticoagulants there is always a proportion of the blood sugar of rabbits in the corpuscles. Using paraffined tubes, relatively less sugar is present in the corpuscles.

Experiments avoiding use of anticoagulants In order completely to avoid the use of anticoagulants, a technique has been developed by which a rapidly excised vein full of blood can be centrifuged and the separated

plasma tied off and analysed at leisure. The disadvantage is that only one determination can be made per rabbit, and it is necessary to do several experiments to obtain average figures which can be relied upon.

The centrifuging of blood in excised veins is, of course, far from new. Our method has been developed quite independently of previous technique, and avoids the complication of anaesthesia. It is as follows: the rabbit, left overnight without food, is killed instantaneously by a blow on the base of the skull, and its inferior vena cava ligatured immediately (within 2 minutes) to prevent sugar mobilisation. Both external jugulars are then laid bare and all branches tied. One is dissected out as rapidly as possible, ligatured at both ends, suspended in oil in a small centrifuge tube and spun. Enough blood for the blood sugar and haematocrit determination is removed from the other jugular by syringe. The first vein is removed from the centrifuge after 5 minutes, the part containing plasma tied off and carefully wiped. The plasma is then emptied into a small tube containing a suitable quantity of potassium oxalate and the plasma sugar determined forthwith. The whole operation, from the death of the animal to the beginning of the plasma sugar determination, takes on the average 20 to 25 minutes, depending on the varying position and number of small veins which have to be tied.

Using this technique, 21 experiments have been done on normal rabbits. Of these, only two showed no reducing substance in the corpuscles, three showed less than 01 p.c. of reducing substance (calculated as glucose per 100 c.c. corpuscles), seven less than 02 p.c. and more than 01 p.c., four between 02 p.c. and 03 p.c., two between 03 p.c. and 05 p.c., while three gave values of 062, 070 and 070 p.c. The haematocrit values lay between 21 p.c. and 37 p.c. corpuscles. From the grouping of the figures, it would appear probable that the last three corpuscle values are abnormally high, the first of these animals is clearly anaemic (haematocrit value = 21 p.c.). From subsequent results it will be seen that the rabbit's corpuscle readily becomes more permeable to glucose, and a few high figures are perhaps to be expected. The results of the jugular vein experiment with normal rabbits are summarised in Table II. The highest blood sugar value obtained was 137 p.c.

TABLE II.

No. of Expts	Between 08 p.c. and 10 p.c.	10 p.c. and 11 p.c.	11 p.c. and 12 p.c.	Above 12 p.c.
Blood sugar	5	6	4	6
No. of Expts	Between 09 p.c. and 01 p.c.	01 p.c. and 02 p.c.	02 p.c. and 03 p.c.	Above 03 p.c.
Corpuscle sugar	5	7	4	5

In three of these determinations Calvert's micro-method for blood sugar was used. In two of the experiments quoted saline was injected two hours before the determinations, which gave values of 015 p c and 021 p c sugar in the corpuscles. In another experiment, 10 c c of 2.5 p c glucose solution were injected subcutaneously a quarter of an hour before the operation. The blood sugar in this case was 168 p c, and the corpuscle sugar 020 p c.

It is probable that the figures in this table give a more accurate picture of the distribution of sugar between plasma and corpuscles in normal fasting rabbits than any previously published. Only a very small quantity of reducing substance is present in the corpuscles, the average figure for their sugar content being 022 p c or, omitting the three highest figures, 017 p c. Outside the corpuscle membrane the concentration of sugar is at least six times as great as it is within it, the actual sugar (*i.e.* plasma sugar) concentration in contact with the tissues, given an average blood sugar value of 11 p c and a hæmatocrit figure of 35, being just over 15 p c, as against the 022 p c corpuscular content.

When the values shown in Table II for corpuscle sugar are plotted against the blood sugar values, a curve is obtained (Fig 1 A) which appears to indicate that the corpuscles *in vivo* have a threshold for sugar at about 0.8 gm (calculated as glucose) per 100 c c blood. Only when the blood sugar is very low is the corpuscle free from reducing substance. As the blood sugar rises the sugar content of the corpuscle appears to rise also, but more slowly. In no case, however, even when anticoagulants are used, as is shown in Table I, has it been found that the corpuscle sugar content equals, or nearly equals, that of the plasma.

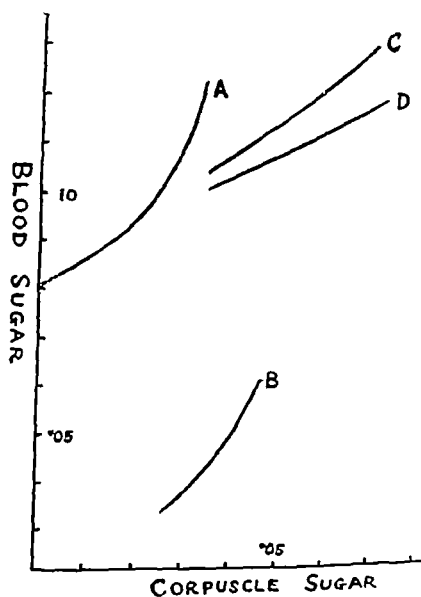


Fig 1 A Normals
B Insulin given
C Insulin (inactivated) given.
D Peptone given.

Effect of insulin on the distribution of "sugar" between plasma and

corpuscles We then investigated the condition existing during the hypoglycæmia following insulin convulsions. One specimen of commercial solid insulin hydrochloride, and another of the liquid preparation were used. A suitable dose of insulin was given to the animal, which was kept under observation until preconvulsive symptoms were noticed, whereupon it was dealt with exactly as described before, and the plasma and whole blood sugar determined in the blood from the jugular veins.

The figures we have obtained indicate that when the hypoglycæmia is approaching the convulsive level, the corpuscular content of reducing substance is not falling with that of the blood, but has risen above the normal value. In fact corpuscular and plasma sugar approximate to the same level. The results of 12 experiments are summarised in Table III.

TABLE III. (Insulinised rabbits)

	Between 0.3 p.c. and 0.4 p.c.	0.4 p.c. and 0.5 p.c.	0.5 p.c. and 0.6 p.c.	Above 0.6 p.c.
No. of expts.				
Blood sugar	3	3	4	2
	Between 0 p.c. and 0.2 p.c.	0.2 p.c. and 0.3 p.c.	0.3 p.c. and 0.4 p.c.	0.4 p.c. and 0.5 p.c.
Corpuscle sugar	1*	3	2	4
				0.5 p.c. and 0.6 p.c.
				2

* Preconclusive symptoms not observed. Killed three hours after injection.

Thus, of the 12 animals used, one showed traces only of reducing substance in the corpuscles, three showed an equal distribution between plasma and corpuscles, and the rest showed quite appreciable amounts of reducing substance in the red cells. Either the corpuscles had become more permeable to sugar, or a non-reducing precursor in the interior of the corpuscle had been broken down to give a reducing substance. The former seems the more likely explanation. The curve (*B*) in Fig. 1 has been drawn from the figures given by 11 rabbits, and would indicate that if there is a threshold level of blood sugar in the normal rabbit, below which no reducing substance is present in the corpuscles, insulin reduces the threshold very markedly.

Glucose still present in blood at the convulsive stage of insulin hypoglycæmia. It has been suspected for some time that possibly a large fraction of the reducing substance present in blood is not glucose. Winter and Smith(s) state that blood-filtrates from rabbits rendered hypoglycæmic with insulin do not show any reducing power when the Wood-Ost reagent (which reacts easily with small concentrations of glucose) is used. Our results up to this point seemed capable of explanation on the supposition that the reducing substance which we found inside the corpuscles, and which even rose in amount during hypoglycæmia,

was not glucose at all. We thought it worth while to ascertain as far as possible whether *any* of the reducing substance in whole blood, which we were estimating when the rabbits were severely hypoglycæmic, was glucose.

The blood of four rabbits in insulin convulsions was collected. Hagedorn and Jensen's method gave, for the mixed blood, a blood sugar of 0.35 p.c. Through the kindness of Mr H. F. Holden, who precipitated the proteins and concentrated the filtrates for us by a method recently elaborated by himself, we obtained a liquid which gave a Wood-Ost value of 0.32 p.c. "glucose," and a Hagedorn and Jensen value of 595 p.c. We are thus able to confirm Winter and Smith to the extent that the Wood-Ost reagent gives a markedly smaller value for the blood sugar in hypoglycæmic blood than does the Hagedorn and Jensen method, though we did not observe complete absence of reducing power towards the former reagent.

The concentrated filtrate was precipitated with phenyl hydrazine in acetic acid solution, and a crude osazone of m.p. 167° was obtained. On recrystallisation twice from aqueous alcohol, and once from aqueous pyridine, the m.p. in a sealed tube rose to 193° – 195° . When this was mixed with an equal quantity of pure glucosazone (m.p. 205° corr.) it gave a m.p. of 199° – 202° . In crystalline form it resembled glucosazone. This would indicate that the osazone obtained from the blood filtrate was a mixture of glucosazone with smaller quantities of another osazone. A combustion was not attempted.

A similar blood filtrate from normal rabbit's blood gave by Wood-Ost a glucose value of 0.90 p.c. and by Hagedorn and Jensen 1.109 p.c., and an osazone prepared from it in the same way gave a m.p. (crude) of 175° , which was raised to 200° by the same methods of crystallisation. By admixture with an equal quantity of glucosazone its m.p. was raised about a degree.

The effect of inactivated insulin and other substances on sugar distribution in the blood in vivo. Insulin, inactivated either by heating at 100° for 10 minutes with *N* sodium carbonate, or at 37° for 90 minutes with *N*/10 caustic soda (Dudley⁽⁸⁾) and neutralised, gave an effect similar to that of active insulin on injection into a starving rabbit, i.e. changes in the permeability of the red cells appeared to follow. The six experiments were done with jugular blood in the manner described above, the rabbits being killed some three hours after the injection. An average corpuscular value of 0.59 p.c. sugar was found. Curve C (Fig. 1) compiled from the six figures given in Table IV indicates that although the red

cell threshold for sugar does not appear to have been altered, the permeability, as judged by the content of reducing substance when the blood sugar is raised, is distinctly increased. In none of these rabbits were abnormal symptoms of any kind observed, nor was the blood sugar lowered.

TABLE IV (Inactivated insulin)

Blood sugar	Plasma sugar	Hæmatocrit	Corpuscle sugar
095	127	34.5	038
111	146	30.5	032
119	136	30.5	081
122	139	30.5	083
136	164	30.0	070
138	188	35.5	048

TABLE V (Peptone)

102	139	32.0	023
102	138	38.0	043
110	129	33.0	072
111	146	35.5	048
124	154	34.0	067

Since inactivated insulin, with chemical properties approaching those of a proteose, gave this corpuscular effect, commercial "peptone," of which certain brands contain much proteose, was tried. Witte's peptone was used in three, and Bacto peptone in two experiments, 15 mg peptone per kilo body weight being dissolved in saline and injected subcutaneously. The results are shown in Table V. There was an average corpuscular content of 0.50 p.c. sugar.

Discussion. From the results recorded above it would appear that the red blood corpuscle of the rabbit is a cell which normally *in vivo* contains only a very small amount of reducing substance, or glucose, but that its permeability to reducing substances may be increased by a variety of agents. The experiments also give some support to the view that "insulin" contains a thermostable substance which is able to change the permeability of the corpuscular membrane to sugar, and which is independent of the hypoglycæmic factor.

Häusler and Loewig(10), in a preliminary note, have stated that insulin causes permeability changes in certain body cells. These authors find that when glucose is added to ground-up tissue from ox arteries and the whole centrifuged, the amount of reducing substance remains constant in the supernatant fluid, if insulin be added, however, the sugar in the fluid diminishes, indicating increased permeability of the artery cells. They obtain the same results with washed red blood corpuscles, controls indicating that glycolysis, or mere swelling of the cells, did not account for the phenomenon. Although, in our opinion, experi-

ments with washed corpuscles or with oxalated blood are of doubtful value in throwing light on conditions *in vivo*, yet Häusler and Loewi's results in conjunction with the still more recent results of Secker(11) must be taken as indicating the presence in insulin of a factor capable of inducing permeability changes in body cells *in vitro*. Neither of these authors seems to have done controls with "inactivated" insulin, i.e. insulin no longer capable of producing hypoglycæmia when injected into rabbits, but otherwise changed as little as possible. It seems to us likely (and this is supported by unpublished experiments of one of us in collaboration with Mr A. Carruthers) that most, if not all, specimens of insulin contain more than one physiologically active factor. Experiments (Kay and Smith(12)) on the blood volume of rabbits following injection of insulin point in the same direction. Secker(11) has shown that, in presence of calcium, guanidine has a similar effect to insulin on the permeability of the erythrocyte of the ox or sheep *in vitro* and our experiments would indicate that injected peptone can bring about permeability changes *in vivo* similar to those produced by insulin.

Until a pure substance of definite chemical composition can be isolated, it is clear that the name "insulin" cannot be precisely used, and may cover a number of factors beside the hypoglycæmic one. It seems reasonable to endeavour to relate other possible physiological properties of a preparation of insulin with its effect on the blood sugar level by controls with inactivated material. Although this would automatically be done with an enzyme preparation, it has almost invariably been neglected hitherto in work with insulin.

Whether or not the amount of reducing substance in the corpuscle of the normal rabbit—0.02 p.c. reckoned as glucose—can be taken to be the amount normally present inside the cells of other tissues remains for the time being no more than an interesting speculation.

SUMMARY

1 In the blood of the normal rabbit only a relatively small proportion of the reducing substance is present in the corpuscles.

2 Addition of anticoagulants to shed blood alters fundamentally the permeability of the corpuscular membrane to reducing substance.

3 The amount of the reducing substance in the rabbit's corpuscle *in vivo* is both relatively and absolutely increased by previous injection of insulin, "inactivated insulin" and peptone solutions.

4 It is almost certain that glucose is still present in rabbit's blood at the stage of hypoglycæmia at which convulsions occur.

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THE RELATION OF SECRETIN FORMATION TO THE
ENTRANCE OF ACID CHYME INTO THE SMALL
INTESTINE—THE PROPERTIES OF SECRETIN
BY J MELLANBY AND A ST G HUGGETT

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In a previous paper⁽¹⁾ the hypothesis was put forward that the metabolism of the pancreatic enzymes is under the control of the vagus nerves, whilst secretin causes the cells of the pancreas to produce a copious flow of sodium bicarbonate (14 N) which carries the pancreatic enzymes with it. On this hypothesis, secretin ensures the presence in the intestine of an adequate supply of sodium bicarbonate to preserve the neutrality of the intestine during the action of the pancreatic and intestinal enzymes. According to Bayliss and Starling⁽²⁾ secretin is derived from prosecretin by the action of acid, and prosecretin exists only in that situation where it is in a position to be acted upon by the acid chyme and to discharge into the blood the substance which acts as a timely stimulus to the pancreatic cells. As a corollary to these statements the secretion of pancreatic juice, produced by secretin, has been causally connected with the secretion of hydrochloric acid by the gastric mucosa. In so far that apparently normal intestinal digestion may be associated with the complete absence of hydrochloric acid from gastric juice, it follows from this hypothesis that the vagus plays the dominant rôle, whilst the secretin mechanism may be adjuvant, but is not essential, to pancreatic secretion. This conclusion, however, is difficult to reconcile with the demonstrable facts of pancreatic secretion. Vagal pancreatic juice though rich in enzymes is extremely scanty in quantity and after prolonged vagal stimulation the cells of the pancreas show marked signs of exhaustion. Secretin juice, on the other hand, although relatively poor in enzymes compared to vagal juice, is secreted in copious quantities, and after a long period of secretion the cells of the pancreas show no sign of exhaustion under the secretin stimulus. Therefore, in order to elucidate these difficulties, and more particularly to reconcile the facts of normal pancreatic digestion with achlorrhya, the distribution of prosecretin in the alimentary canal, the assumed existence of prosecretin and the properties of secretin were investigated.

The distribution of prosecretin in the alimentary canal Bayliss and Starling⁽³⁾ found that secretin extracts of the duodenum were more effective in causing a flow of pancreatic juice than those of the jejunum, whilst those of the ileum were inactive. Similarly, Lalou⁽⁴⁾ made secretin extracts from the mucous membrane of various parts of the alimentary canal and compared their secretin contents on the same dog. He found that the extract of the duodenum contained approximately ten times as much secretin as the corresponding extract from the ileum and eighty times as much as the stomach extract. In the investigation of the distribution of secretin in the small intestine, the anatomical division of that portion of the gut into duodenum, jejunum and ileum was not followed. It appeared more reasonable to estimate the relative quantities of secretin in those portions of the gut in which digestion and absorption varies to the greatest extent. For this reason the secretin contents of the mucous membrane of the stomach (fundus and pylorus), small intestine (upper, middle and lowest third) and ascending colon were estimated. These experiments were carried out at the beginning of the investigation when it was assumed that secretin exists in the inactive form (prosecretin) in the mucous membrane. The extracts were therefore made by 2 p.c. HCl in the method of Bayliss and Starling. The results are, however, comparable to those obtained with other extractives of secretin.

The mucous membrane was scraped from the alimentary canal of a goat within one hour of its death. 20 gm. of the mucous membrane from various portions, after being well ground up with sand, were boiled with 40 c.c. of HCl 2 p.c., neutralised and filtered according to the accepted method for making secretin, 4 c.c. of each of these filtrates was injected in turn into the femoral vein of an anaesthetised cat (urethane 1.5 gm. per kilo) and the quantity of resulting secretion from the pancreatic duct was measured.

Part of gut taken	Pancreatic juice secreted in c.c.
Fundus of stomach	Nil
Pylorus of stomach	Nil
Upper third of small intestine	2.75
Middle third of small intestine	2.6
Lowest third of small intestine	0.5
Ascending colon	0.2

These figures confirm to some extent the observations of Bayliss and Starling and of Lalou, on the distribution of prosecretin. The fact, however, which we desire to emphasise is that prosecretin exists in practically undiminished quantities in two-thirds of the small intestine.

of the goat It is evident, therefore, on the acid hypothesis of secretin formation, that although hydrochloric acid of the gastric juice may be an important factor in the formation of secretin from prosecretin, yet other substances produced in the intestine may enter into the mechanism. In this connection, amino acids produced from the digestion of protein in the duodenum and jejunum were considered as a possible source of acid This hypothesis appeared to be verified by certain experimental results Active secretin extracts were obtained by boiling the intestinal mucosa with water containing glutamic acid, leucine, tyrosine, etc It appeared, therefore, that the occurrence of prosecretin in considerable quantities in the mucous membrane of the intestine well below the action of acid chyme might be appreciable on the hypothesis that amino acids could actively participate in the reaction As an extension of the hypothesis, it is evident that fatty acids obtained from the digestion of fat by the first secreted pancreatic lipase might also participate in this mechanism since, as Moore and Rockwood(16) have shown, a meal of fat induces a weak acidic reaction throughout the greater part of the small intestine in the dog In order to test the accuracy of the hypothesis that amino acids and organic acids might convert prosecretin into secretin and thus supplement the initial excitatory action of the acid chyme, the capacities of a variety of solvents to extract secretin from the intestinal mucosa were determined

The existence of prosecretin According to Bayliss and Starling(5) secretin is formed from prosecretin by a process of hydrolysis They found that mineral acids were more effective than organic acids in producing this reaction and that a weak acid like carbonic acid was ineffective These results were confirmed and extended by Camus(6) who, in addition to carbonic acid, found that boric acid was incapable of producing secretin from its precursor in the intestinal mucous membrane In marked contrast to these results, a large number of observers have produced active extracts of secretin from the intestinal mucous membrane by solutions containing substances other than acids Among such substances may be mentioned sodium oleate (Fleig(7)), chloral hydrate (Falloise(8)), ethyl alcohol (Fleig(9)), sodium chloride (Delezenne and Pozerski(10)), Witte's peptone (Gley(11)), cane sugar, glycine, urea and soaps (Frouin and Lalou(12)) In order to test the hypothesis that the precursor of secretin (prosecretin) exists in the mucous membrane of the small intestine and that this precursor is effectively hydrolysed by acids only, and thereby converted into secretin, the following experiment was carried out

The mucous membrane of the upper two thirds of the small intestine of a pig was ground up with sand and divided into ten equal portions. Each portion was boiled with twice the quantity by volume of (1) 75 p c alcohol, (2) 75 p c acetone, (3) 5 p c NaCl, (4) 7 p c NaCl, (5) H_2O , (6) 0.5 p c. NaOH, (7) 1 p c NaOH, (8) 2 p c NaOH, (9) phosphate pH 6.5 and (10) phosphate pH 7.5. After neutralisation (if necessary) and filtering, the filtrates were tested for secretin on a cat anaesthetised with urethane (1.5 gm per kilo). The number of drops of pancreatic juice and the rate of secretion were determined in each case after the intravenous injection of 2 c c of the filtrates into the femoral vein.

Drops	75 p c alcohol		75 p c acetone		5 p c. NaCl		7 p c NaCl		H_2O	
	m	s	m	s	m	s	m	s	m	s
2		47	1	30		47	1	40	1	55
4	1	16	2	9	1	20	2	25	2	45
6	1	57	3	7	1	53	3	6	4	5
8	2	43	4	2	2	24	3	58	5	35
10	3	15	4	47	2	58	4	46	7	3
12	3	46	5	33	3	36	5	36	8	56
16	4	59	7	20	5	23	8	6	16	0
20	6	16	9	49	7	45	12	10		
24	7	47	14	57	11	9	—	—	—	—
28	9	45	—	—	—	—	—	—	—	—

Drops	0.5 p c. NaOH		1 p c NaOH		2 p c NaOH		Phosphate pH 6.5		Phosphate pH 7.5	
	m	s	m	s	m	s	m	s	m	s
2	1	36	2	5	1	25	1	50	1	55
4	2	25	3	31	2	20	2	40	2	53
6	3	9	4	52	3	35	3	55	4	5
8	3	51	6	54	4	53	5	30	5	38
10	4	48	10	52	6	35	7	50	8	10
12	5	49	17	30	9	40	13	20	13	0
16	8	18	—	—	—	—	—	—	—	—
20	13	12	—	—	—	—	—	—	—	—

It may be observed that the filtrates from the 75 p c alcohol extract contain only about 50 p c alcohol owing to the quantity of water contained in the intestinal mucosa which is subjected to extraction. Similar remarks apply to the acetone extract. The results indicate the apparent marked diversity of solutions which can be used as effective extractives of secretin. The efficiency of dilute alcohol for the preparation of secretin is well illustrated. Within 10 minutes of the injection of 2 c c of alcoholic secretin into the blood stream, the pancreas secreted 2 c c of juice. In order to emphasise the efficiency of dilute alcohol as an extractive for secretin, an experiment was carried out in which dilute alcohol and 2 p c HCl were compared directly. The results obtained are given below (p 126).

The result emphasises the marked superiority of dilute alcohol over acid for the preparation of secretin extracts from the intestinal mucous membrane. The comparative records of the two experiments show that acid is a less effective extract for secretin than alcohol (50 p c), acetone (50 p c), 5 p c NaCl and 7 p c NaCl.

Drops	75 p c alcohol		2 p c HCl	
	m	s	m.	s
2		15	1	0
4		50	1	25
6	1	12	1	50
8	1	36	2	16
10	2	6	2	46
12	2	50	3	27
16	4	13	5	25
20	5	43	8	10
24	7	20	—	—
28	9	14	—	—
32	11	0	—	—
Total juice	22 c c		14 c c	

It is evident that hydrochloric acid possesses no specific capacity for the preparation of secretin extracts from the intestinal mucosa. The fact that a comparatively strong solution of alkali (2 p c NaOH) is able to extract secretin is a definite proof against the hypothesis that pro secretin is hydrolysed by acids with the formation of secretin. The experiments establish the fact that secretin is contained in a preformed condition in the mucous membrane of the upper two-thirds of the small intestine, and that this secretin is soluble in water, and is stable in dilute solutions of acid, acetone and alcohol.

Properties of secretin According to the observations of Bayliss and Starling and W. A. Osborne(13), secretin may be regarded as a simple substance since it is stable when boiled in dilute acid or alkaline solutions and may be dialysed. Further, according to these observers, it is not precipitated from solution by tannic acid, a fact which differentiates it from a protein, alkaloid or diamino acid. On the other hand, it may be removed from solution by salts of the heavy metals. Dale and Laird(14) showed that the precipitation of secretin by mercury salts could be utilised to purify secretin preparations. It has been stated(15) that secretin appears to be an amine derived by decarboxylation of an amino acid, but the evidence in support of this statement is not available.

Solubility From the foregoing experiments it is evident that secretin in the intestinal mucosa is freely soluble in water, and is comparatively stable when boiled in solutions of sodium chloride (5 p c), phosphate (pH 6.5 and 10.5), dilute acids (2 p c HCl), dilute alkali (1 p c NaOH), dilute alcohol (50 p c) and dilute acetone (50 p c).

Action of enzymes Bayliss and Starling found that secretin is rapidly destroyed by trypsin. This fact would appear to indicate that secretin is a protein or polypeptide capable of being hydrolysed by this enzyme. Secretin, however, is readily destroyed by pepsin and since rapid peptic digestion proceeds only as far as proteoses it follows that

secretin must belong to that class of proteins. Alternatively, secretin may be a relatively simple substance which owes its solubility to its association with a proteose, and when this association is broken by digestive enzymes the labile secretin is destroyed. In this connection the action of intestinal intracellular enzymes is of practical importance. It is well known that secretin solutions made in a routine manner show considerable variations in activity. The circumstances underlying these variations were determined. An important factor was the duration of the interval between the death of the animal and the extraction of the intestinal mucosa. The following experiment illustrates this statement and indicates the cause of the variation.

The mucous membrane from the small intestine of a pig was divided into three equal portions and suspended in (a) H_2O , (b) 2 p.c. HCl, and (c) alkaline phosphate (pH 10.5). The suspensions were left at $37^\circ C$ for 1 hour. After this time secretin solutions were prepared from them in the usual way by boiling, etc. The alkaline phosphate solution showed a slight amount of activity; the water and 2 p.c. HCl extracts were completely inactive.

It is evident, therefore, that secretin is destroyed by the enzymes in the intestinal mucosa, rapidly in a neutral or acid medium and more slowly in an alkaline medium.

Precipitation (a) Ammonium sulphate The precipitation of secretin solutions by Am_2SO_4 gives characteristic results. The precipitate formed by half saturation with Am_2SO_4 contains no secretin, the precipitate obtained by saturation of the filtrate from this mixture with Am_2SO_4 contains all the secretin of the original solution.

(b) *Alcohol* added to a secretin solution to the extent of 85 p.c. at $0^\circ C$ produces a partial precipitation of secretin.

(c) *Tannic acid* From the foregoing description it is evident that secretin is soluble in water, is not destroyed by heat ($100^\circ C$), is not precipitated by half saturation but is precipitated by full saturation with Am_2SO_4 . Further, it is rapidly destroyed by trypsin, pepsin and the intracellular enzymes of the small intestine. All these properties indicate that secretin is a secondary albumose. Bayliss and Starling, however, suggest that secretin belongs to a much simpler class of substances, since it is not precipitated by tannic acid. Detailed experiments were therefore carried out to analyse this precipitation. The complete precipitation of proteins by tannic acid is a matter of considerable difficulty. The reaction of the fluid must be made slightly acid and the degree of acidity and the required amount of tannic acid varies with every protein solution. These difficulties have been recognised by Almén, who has made a tannic acid reagent con-

taining acetic acid and alcohol which he states to be a more effective precipitate than ordinary tannic acid solutions. The activities of a secretin solution before and after precipitation by tannic acid and Almén's reagent are shown in the following figures which were all obtained from the same cat

Drops	Original secretin solution		Filtrate after ppt. by tannic acid		Filtrate after ppt. by Almén's reagent	
	m	s	m	s.	m	s.
5	1	55	3	36	1	55
10	3	0	5	0	3	26
15	4	1	6	38	5	35
20	5	8	8	40	8	29
25	6	1	12	20	14	45
50	11	30	—	—	—	—
75	22	23	—	—	—	—
Total juice	43	c c	16	c c	14	c c

It is evident that tannic acid removes considerable quantities of secretin from solution, the amount removed being comparable to the proportion of protein precipitated. The results certainly offer no evidence in favour of the hypothesis that secretin is not a protein.

Colloidal iron and colloidal gold. Secretin is not precipitated from solution by either positively or negatively charged suspensoid colloids. The precipitation of colloidal iron in a secretin solution does not diminish the secretin activity of the resulting filtrate. A similar fact is true for colloidal gold precipitation. From this it may be inferred that secretin is electrically neutral when dissolved in water.

The action of the products of peptic digestion on pancreatic secretion. The foregoing facts suggested that secretin is a secondary albumose formed in gastric digestion which, when absorbed into the blood, stimulates the pancreas to secrete. This hypothesis, if correct, would correlate gastric and pancreatic functions. No experimental evidence was obtained in favour of it. The intravenous injection of proteose solutions and peptic digests in all stages of digestion into cats never produced any secretion from the pancreas, although control experiments with secretin evoked well-marked pancreatic secretion. The hypothesis was directly negated by an experiment with a foetal goat obtained from the uterus two weeks before full term. The small intestine of this foetus, when extracted with 2 p c HCl in the usual way for the preparation of secretin, produced a well-marked secretion of pancreatic juice when injected into a cat. It is evident, therefore, that secretin has no direct relation to gastric digestion unless the secretin in the foetal mucous membrane is derived from the secretin in the maternal goat's blood and stored in it during development.

Secretin as a primary amine Secretin is described by Robertson⁽¹⁵⁾ as a primary amine derived by decarboxylation of an amino acid. This statement was tested by submitting a secretin solution to the action of nitrous acid. No destruction of secretin occurred. Since nitrous acid decomposes primary amines with the evolution of nitrogen, it is evident that the statement made by Robertson is inaccurate.

Discussion

The experimental results show that considerable quantities of secretin exist in the mucous membrane of the upper two-thirds of the small intestine. There is no evidence that hydrochloric acid is necessary for the formation of secretin from an assumed precursor in the cells of the intestinal mucosa, nor is there any evidence that this assumed precursor exists only in that portion of the small intestine in which it is in a position to be acted upon by acid chyme. The experimental facts do not afford any basis for the assumption that pancreatic secretion and gastric hydrochloric acid are causally connected. Further, the results show that the secretin mechanism for the production of pancreatic juice may function in complete gastric achlorrhya. An analysis of the properties of secretin shows that it is soluble in water, is not destroyed by heat (100°C), is not precipitated by half saturation with Am_2SO_4 , but by full saturation with this salt. Further, secretin is rapidly destroyed by pepsin, trypsin and the intracellular enzymes of the small intestine. All these properties indicate that secretin is a secondary albumose or is intimately associated with a secondary albumose. Experiments with various protein fractions obtained by peptic and tryptic digestion indicate that secretin is not derived from the digestion of protein food-stuffs. This conclusion was confirmed by the fact that the small intestine of an immature foetal goat, taken directly from the uterus, contained large quantities of secretin.

SUMMARY

1 Secretin exists in a preformed state in considerable quantities in the mucous membrane of the upper two-thirds of the small intestine. A relatively small quantity of secretin may be extracted from the lowest third of the intestine.

2 Secretin contained in the mucous membrane of the intestine is soluble in water, and is relatively stable in dilute solutions of acid, alkali, alcohol and acetone. It may therefore be obtained by extracting the mucous membrane of the upper two-thirds of the small intestine with

water, 2 p c NaOH, phosphate solutions (pH 7.5, 6.5), 2 p c HCl, 75 p c alcohol and 75 p c acetone

3 There is no evidence that gastric hydrochloric acid converts a precursor (prosecretin) in the duodenal mucous membrane into secretin and thereby excites a flow of pancreatic juice

4 Secretin possesses all the properties of a secondary albumose, being soluble in water, precipitated on full saturation with Am_2SO_4 and destroyed by pepsin, trypsin and the intracellular enzymes of the small intestine

5 Secretin is not derived from the alimentary digestion of protein, since it is present in considerable quantities in the small intestine of the foetus

6 There is no evidence that the chemical group to which secretin owes its activity is of the nature of a primary amine

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ON THE NATURE AND SIGNIFICANCE OF VAGUS ESCAPE BY R J S McDOWALL

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THE escape of the heart from vagus inhibition has generally been attributed either to an outburst of energy stored during rest or to a fatigue of the nerve endings Hill and Barnard(1), however, showed that compressing the abdomen when the heart was stopped, and thus producing a rise of pressure on the right side of the heart, might cause escape, and more recently Bainbridge(2) found that increase in the pressure in the right heart by venous injection increased the rate of the heart beat. Since this did not occur after section of the vagus he considered that the effect was a reflex one by way of the vagus, although he did not exclude entirely increased sympathetic action. It seemed then possible that increased blood pressure in the right side of the heart, caused by increased venous pressure, might play an important part in vagus escape, and this question I have investigated. All the experiments were made on cats, anaesthetised in the first instance with ether and later with choralose, and in order to make the conditions as near as possible to those occurring ordinarily in life nearly all my experiments have been made of escape from partial, not complete, inhibition. Further I have discarded all experiments in which there was indication of shock.

The effect of venous pressure on vagus escape

In these experiments venous pressure was recorded by a method which I have described elsewhere(3). The heart rate was recorded by an ordinary mercurial manometer. It was realised, however, that strictly speaking this may not always be correct, but with high blood-pressures it has been found on checking that the waves were reasonably accurate records of left ventricular beats.

Raised venous pressure The rise of venous pressure which occurs on stimulation of the vagus in an animal with good circulation is as striking as the fall of arterial pressure (Fig 1), and this fact together with the observation that after a brief period of vagal stimulation the heart is markedly accelerated was really the starting point of this investigation. Such a rise can also be simply produced by injecting fluid,

such as gum-saline, into the veins. When the strength of stimulus was increased to an extent which just prevented the escape, the escape was

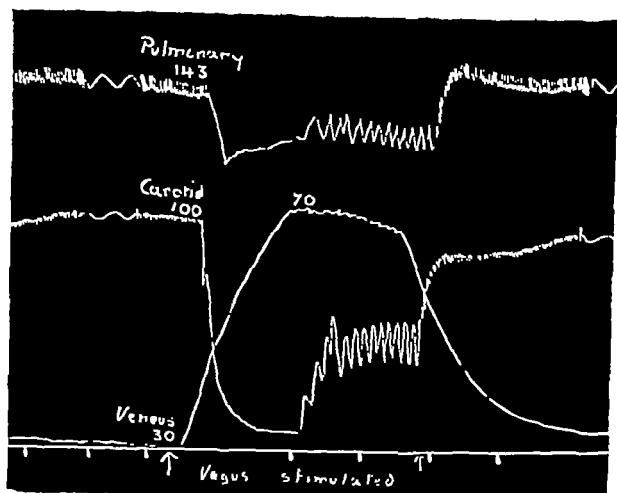


Fig 1 1 mm of tracing = 3 mm pressure. Pulmonary and venous pressure in mm. H_2O , carotid in mm. Hg. Typical result of stopping the heart by stimulating the vagus. Pulmonary and carotid pressures both fall, venous pressure rises abruptly. When the venous pressure has risen to 70 mm. H_2O ventricular escape occurs which brings about a partial recovery of the arterial pressure and a slight decrease of the venous. On shutting off the stimulation (second arrow) there is typical recovery.

readily brought about on the injection of fluid. This occurred even when the fluid injected was at ordinary laboratory temperature, so that apparently the effect of the tension more than counteracted the effect of the cold. The venous pressure could also be raised by compressing the abdomen and vagus escape brought about in this way as pointed out by Hill and Barnard(1). In such procedure, however, the arterial pressure is also raised and if the vagi were intact it might be expected that the rise of aortic pressure would have the opposite effect and slow the heart by means of the depressor reflex. Apparently however as a rule the rise of venous pressure more than counterbalances the effect of the rise of aortic pressure, but in one experiment the compression of the abdomen with the hand brought about a preliminary slowing of the heart which was followed by an acceleration although the blood-pressure had risen to a higher level, indicating that in this instance the rise of aortic pressure caused in the first instance a slowing of the heart which more than counterbalanced the rise of venous pressure (Fig 2).

It was also noted that after raising the venous pressure by compression of the abdomen a strength of stimulus which brought about

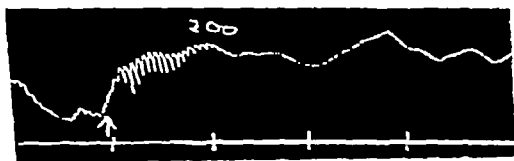


Fig 2 Tracing arterial pressure Abdomen compressed at arrow

vagal inhibition might no longer do so. In such compression of the abdomen it was noted also that the effect became less if the compression was often repeated, while if the venous pressure was already high no further increase could be expected to cause further acceleration. It will of course be understood that what has been said above refers to escape from the minimal stimulus which will bring about inhibition.

Prevention of the rise of venous pressure The rise of venous pressure which occurs in vagal stimulation can readily be prevented by introducing between the venous cannula and the manometer a compensator such as that described by Roberts(4) or a valve. It was found, as seen in Fig 3, that if in this way the venous rise was prevented, vagus escape did not occur but as soon as the compensator was shut off and the venous pressure permitted to rise, vagus escape at once took place. In

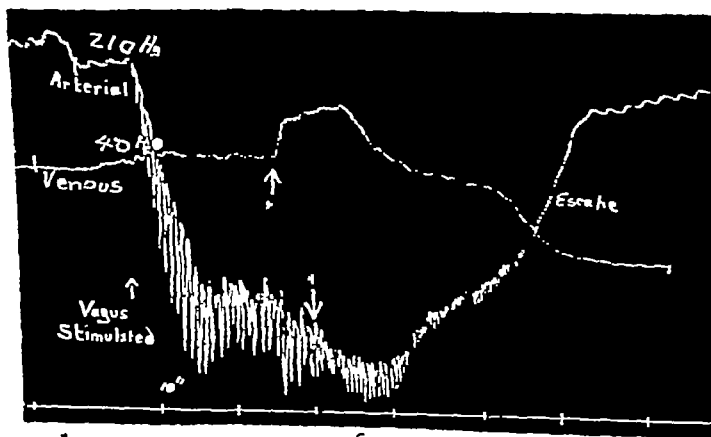


Fig 3 $\times \frac{1}{2}$ Stimulation of vagus begun at first arrow and continued throughout. At the second arrow, which are corresponding points in the two tracings, the venous pressure was allowed to rise and there is at once an increase in heart rate.

carrying out this experiment great care has to be taken to insure that the venous cannula is quite free as the hæmorrhage into the compensator tends to lead to blocking

Lowered venous pressure As I pointed out in a previous paper(5), after severe hæmorrhage the venous pressure becomes so low that appreciable slowing of the heart does not alter the cardiac output per minute, since the filling is increased by the lengthened diastole. For this reason if the venous pressure is sufficiently low, there is little or no change in the arterial and venous pressures, and it is found that in such circumstances vagus escape does not occur. If the hæmorrhage is less severe the escape is delayed appreciably, apparently because a larger rise of venous pressure must occur before the cardio-accelerator mechanism is stimulated sufficiently. In Fig 4 is given an example where the rise of venous pressure in the superior vena cava rose from 26 to 40

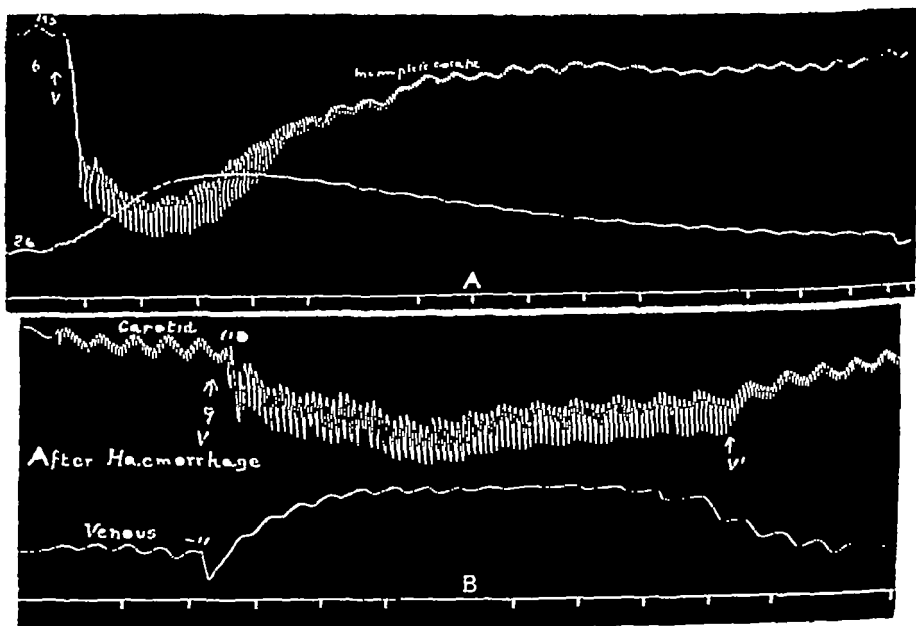


Fig 4 In A in which the venous pressure rises from 26 to over 40 mm there is considerable, but not complete, escape although the vagus was stimulated continuously, commencing at the arrow

B taken from the same animal after hæmorrhage $Pr = 110$, the venous pressure has fallen proportionately very much more. The vagus was stimulated between the arrows there is no appreciable recovery of the normal rate. But it will be noted that the venous pressure never rose above a maximum of zero mm

and a typical escape was brought about, while a few minutes later after severe hæmorrhage which lowered the venous pressure appreciably and on stimulation it never rose above a maximum of zero, no escape occurred. It was also found that in the latter instance the strength of stimulus necessary to bring about even a great slowing of the heart was appreciably less.

The effect of hæmorrhage was also seen if the loss of blood took place during the escape and it was found that in such circumstances the escape tends to disappear and the vagus action to become more effective as the hæmorrhage proceeds (Fig 5). This experiment is not, however, always successful since it depends on a narrow range both of strength of stimulus and degree of hæmorrhage. In experiments controlled by taking the venous pressure at the same time, it was found that the normal rise of venous pressure on vagal stimulation was converted into an enormous fall by the hæmorrhage.

The effect of the strength of stimulus on vagus escape

Hough(6) and others have recorded that vagus stimulation had after a time no effect. Similarly in these experiments after vagal inhibition from which there had been complete escape, it was found that frequently the same strength of stimulation no longer would bring about inhibition. This has been noted even when there has been vagal stimulation without actual escape. It has been pointed out by MacWilliam(7) that during vagal inhibition there is diminished irritability even to subsequent vagal stimulation. In some animals a moderate stimulus allowed a partial escape only, that is although the heart rate recovered to a large extent recovery was not complete, which appeared to indicate that the strength of stimulus used was in excess of the counteracting stimulus brought about by the rise in venous pressure. Even when the escape was complete a strengthening of the stimulus usually brought about further inhibition. It is also observed most clearly that hæmorrhage increased the sensitivity of the animal to vagal stimulation (see Figs 4 A and 4 B). In two animals it was found quite impossible to stimulate the vagus by any strength of current, and this I understand has been with some other workers, e.g. Hough(6), a fairly common experience. In the animals referred to, it was found that the venous pressure was abnormally high, over 100 mm H_2O , and on reducing this by bleeding the reaction of the animal to vagal stimulation became normal. This would appear to show that the venous pressure was so high that no amount of vagal stimulation could counteract it. The cause of this high venous

pressure is not yet clear. The experiments were carried out under conditions similar to scores of others, except that they were done during the heat wave when the animals had been kept and experimented upon

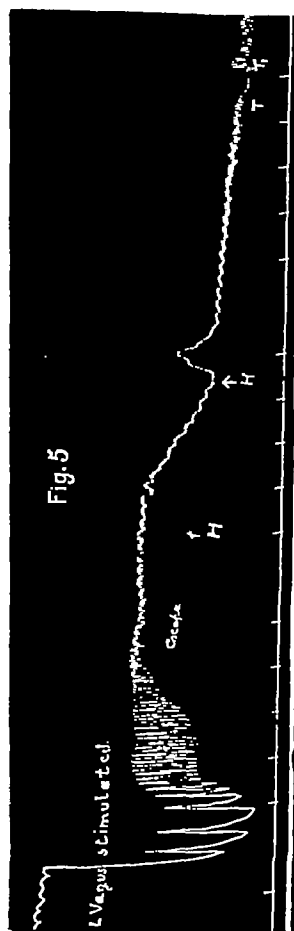


Fig. 5

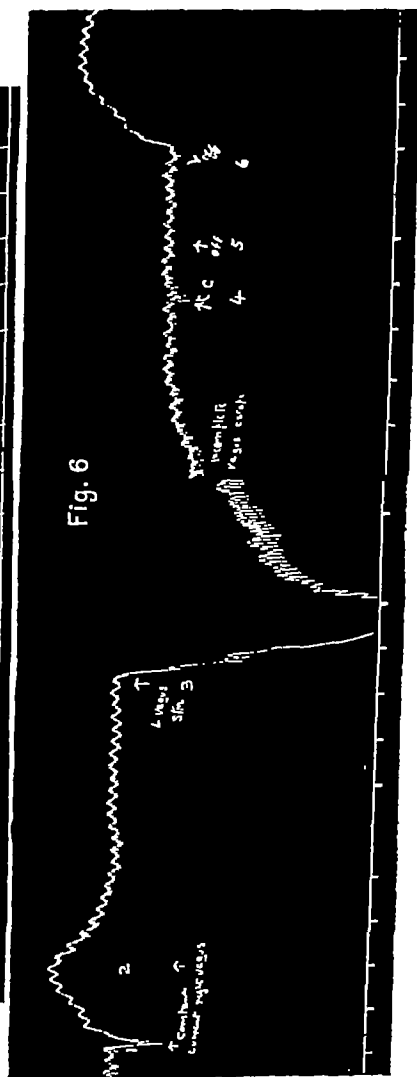


Fig. 6

Fig. 5 Arterial pressure. Effect of hemorrhage on 'vagus escape'. After the hemorrhage, stimulation of the vagus again becomes effective although there has been no alteration in the stimulation itself. From T to T_1 the stimulating current was shut off to show more clearly the effect of vagus stimulation.

Fig. 6 Arterial pressure. At 1-2 constant current was applied to the right vagus to determine the vagus restraint present. At 3 the left vagus was stimulated, incomplete escape occurring. When the test for vagus restraint was applied as before (3-4) although the heart is slowed the tone in the right vagus has disappeared. At 6 the vagus stimulation was shut off.

in rooms over 80° F. It seems possible that the high venous pressure was brought about by the secretion of adrenaline which Cramer (5) has suggested occurs in such circumstances or to cardiac impairment produced at the early stage of the experiment by excess of volatile anæsthetic.

The relation of vagus escape to the nervous control of the heart

Relation to so-called vagus tone In these experiments animals with a marked vagus tone were chosen in order that any diminution in this tone would be more clearly evident. Normally in such animals section of the vagi brings about a very much increased rate of the heart and rise of blood-pressure. If, however, the vagi are cut during vagus escape it is found that there is no further increase in the heart rate. The absence of vagus tone may be shown in other ways. Nervous impulses passing down by the vagus were blocked by passing a constant current through each nerve. The blocking caused an increase in the heart rate similar to that caused by section of the vagi. When, however, conduction was blocked during vagus escape or immediately after it, there was no increase in heart rate (Fig 6). The above experiments show conclusively that stimulation of the vagus brings about a disappearance of the normal vagus tone, even in the nerve which is not being stimulated. It has been clearly seen, however, that the loss of vagus tone is not wholly responsible for the escape as the latter may take place after section of both vagi. Bainbridge(2) could not find evidence of any other afferent path for the acceleration produced by venous injection than by the vagus, but it does not seem at all clear that his experiments were conclusive on this point. As it has been shown by Langley(8) and McDowall(9), using different methods, that afferent impulses pass from thoracic viscera by way of the stellate ganglion, it seems quite probable that impulses concerned in this reflex may also pass by way of afferent sympathetic fibres, and that this must be so is suggested by the marked effect of removal of the stellate ganglia.

Relation to the sympathetic This can be most easily demonstrated by removal of the stellate ganglia. These are most readily exposed by detaching the second rib at its chondro-sternal junction, cutting down each side of the rib, taking care to avoid the intercostal arteries at the infra costal margin, and forcibly dislocating the rib outwards, completing the exposure of the ganglia by blunt dissection. In some of these experiments the decrease of vagus tone was excluded by section of the vagi.

When the ganglia had been removed and the electrodes applied to the peripheral vagus in the neck, it was found that very slight slowing is maintained with little or no change (Fig 7). A very slight escape is sometimes seen but this is not comparable with that seen normally and may be taken to be due to some direct effect of the rise of venous

pressure on the right side of the heart. The only limit, indeed, to vagal inhibition appears to be the life of the animal. The anoxæmia of venous

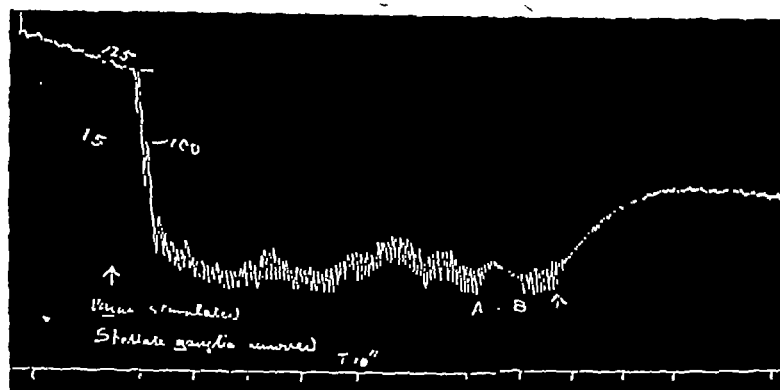


Fig 7 Arterial pressure, vagi cut, stellate ganglion removed, peripheral end of left vagus stimulated between the arrows. No escape. From A to B the stimulating current was shut off temporarily.

congestion, as shown by Bolton(10), brings about an increased permeability of the capillaries and œdema and hence it is found that after stimulation of the vagus for a minute although the arterial pressure may recover, the rise in venous pressure is followed by a marked reduction, such as occurs in hæmorrhage or in histamine shock. Vagal stimulation has been kept up for over an hour during which the blood-pressure fell from 150 to 20 mm of mercury. These results confirm those of Hough who concluded that vagus escape was certainly not due to vagus fatigue. The experiments indicate that stimulation of the sympathetic is as important a factor as loss of vagus tone.

DISCUSSION

It may be reasonably concluded that there occurs during vagus escape a decrease in the action of the cardio-inhibitory centre or what Langley suggests should be called "vagus restraint" rather than "vagus tone," and the stimulation of the sympathetic, which taken together may be called a stimulation of what is best described as the cardio-accelerator mechanism, and from the evidence previously given in relation to venous pressure, it may also be concluded that the means by which the mechanism is stimulated during vagus escape is that which takes place normally in exercise, namely a rise in venous pressure. Three pieces of evidence, namely the relation of venous pressure, of

vagus restraint and of the sympathetic are all the more convincing as they are all quite separate yet are mutually supporting. It could indeed be most readily argued that the most likely thing to bring about such nervous changes under such circumstances as vagal stimulation would be a rise of venous pressure even if it had not been shown that this was brought about.

Although the above results appear to show fairly conclusively that the stimulation of the cardio-accelerator mechanism is the cause of the vagus escape, one other possibility requires careful consideration, namely that the fall of arterial pressure during vagal inhibition might, in accordance with Marey's Law, bring about a quickening of the heart. Were this the case it might reasonably be expected that the reduction of blood-pressure by hæmorrhage would render the animal less sensitive to vagus stimulation, while the reverse is found to be clearly the case. Also it would be anticipated that were vagus escape due to fall of arterial pressure, it would occur whenever the arterial fall occurs, whatever the change of the venous pressure, and this we have seen above is clearly not the case. Further, when an animal was bled during the escape to an extent below that to which it was lowered by the cardiac inhibition, the result was increased slowing rather than quickening. It may then be concluded that the fall of arterial pressure is not an important factor in the production of vagus escape although possibly it may assist. In view of the above and the fact that we know that in exercise a cardiac acceleration may be brought about in spite of a rise of aortic pressure, it seems possible that the application of Marey's Law should be restricted to the operation of the depressor reflex by which there occurs a lessening of the depressor impulses normally passing up from the aorta when there is a fall of arterial pressure, unless it be made also to relate to simultaneous changes in the venous pressure.

The significance of vagus escape The significance of the relation of the rise of venous pressure to vagus escape lies in the fact that it may be considered that such escape indicates the physiological mechanism by which an increased heart rate may be brought about in spite of increased vagus tone or increased reflex stimulation by the depressor nerves. That there must be some antagonism during the rise of aortic pressure in exercise between the depressor and accelerator mechanism is evident.

The significance of vagus restraint From the above facts we are in a position to appreciate the significance of increased vagus restraint which we know is associated with habitual activity in man and in

animals It will be evident that this increase will prevent cardiac acceleration by a rise of venous pressure which would otherwise do so, but this restraint does not appear ever to have been associated with the fact that habitual activity also leads to greater efficiency on the part of the heart muscle The more efficient the cardiac muscle the better the heart can respond to increased filling, and the more capable it is of increasing its output in accordance with the law of the heart Were there, however, no increased vagus restraint, the heart could not take full advantage of this increased efficiency since exercise would bring about excessive acceleration with a shortened diastole and there would be less time for filling Vagus escape, then, by reducing the amount of the acceleration for a given rise in venous pressure makes the heart more capable of increasing its output with a minimum number of beats, presumably with a view to economising the energy of the animal as it is obviously more economical to have a fewer number of efficient contractions than a larger number of less efficient contractions, although both would have the same effect in increasing the cardiac output per minute It is also known that in repeated exercise there is brought about a considerable economy in muscular effort possibly from better coordination which must result in less CO_2 being produced and a less raising of venous pressure which would further supplement the economy of the heart

SUMMARY

Evidence is put forward that vagus escape is sufficiently accounted for by the rise of venous pressure which occurs during vagal stimulation

There is found to be diminution in vagus restraint and stimulation of the sympathetic and this may be spoken of as a local cardio-accelerator mechanism The significance of vagus escape and vagus restraint is discussed on the basis of these results

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THE ACTION OF ADRENALIN AND ERGOTAMINE ON THE UTERUS OF THE RABBIT

By J H GADDUM

(From the Wellcome Physiological Research Laboratories)

WHEN a rabbit's uterus is cut in pieces and tested with ergot alkaloids and adrenalin, according to the technique introduced by Broom and Clark⁽¹⁾ as a test for ergot alkaloids, it gives reactions which are notably consistent over long periods. These reactions have been further considered in the hope of arriving at a more precise idea of their nature.

The action of varying concentrations of adrenalin The rabbits used weighed from 1.8 to 3 kilos. Some of them were pregnant, some were not. The volume of the baths was about 20 c.c. each and they were contained in a water bath similar to that used by Dale for assaying pituitary extracts. The oxygen was delivered in small bubbles through a fine jet. The tension on the muscle was about 1 gram weight. About 1 mg. of Sandoz ergotamine was weighed out each morning and dissolved in water to a strength of 1/5000 by the addition of 2 drops of 3 p.c. acetic acid. The adrenalin used was Burroughs, Wellcome and Co.'s 1/1000 solution. The drugs were diluted with water and added to the bath in volumes which varied from 0.15 to 1 c.c. The Ringer solution used was NaCl 9 p.c., KCl 0.42 p.c., CaCl_2 0.24 p.c., NaHCO_3 0.5 p.c., glucose 1 p.c.

According to Clark a uterine horn should be cut into pieces about 1 cm. long and each piece should be divided in the plane of the mesentery and secured in the apparatus by its anti-mesenteric border.

The value of this was confirmed. At the attachment of the mesentery the uterus seems unable to relax. When pieces of large uteri were divided into four quarters, the quarters free of mesentery worked well and the others did not. The pieces of uterus kept well in an ice box on glass, but after they had been in the bath some time they began spontaneously to produce a series of short-lived contractions returning always to the same base line. This rhythm was particularly marked in the pregnant uteri. The addition of adrenalin had the effect of raising the base line.

and the height to which it was raised was thought to be the best indication of the size of the contraction

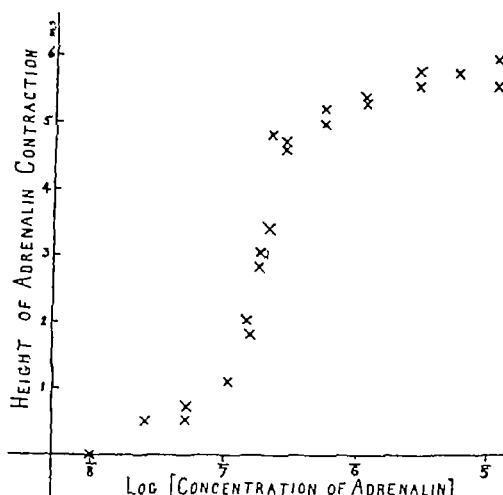


Fig 1. See Text. Ordinates show double the actual shortening of the muscle.

This gave fairly constant readings independently of the spontaneous rhythm. In Fig 1 the height so measured is plotted against the logarithm of the concentration of adrenalin. Shackell has obtained curves of similar form for the effect of adrenalin on arterial rings(2) and also by plotting the percentage mortality among a number of individuals against the dose of toxic substance(3). These results have been confirmed in experiments with a number of different drugs in this laboratory, and a paper on the subject by J W Trevan is in preparation. The form of the curve is that which would be obtained on the assumption that the drugs are acting on a number of units whose susceptibility is distributed about a mean in accordance with a probability curve—it is the curve of the integral of the normal distribution.

It was thought that if the mechanism producing changes in tone was quite distinct from that producing the rhythmic contractions, it might be possible to demonstrate a difference in the shape of the tracings or of the above curves when an isometric lever was used. Tracings obtained with such a lever were directly compared with those obtained with an isotonic lever. They were very similar.

Adrenalin is very easily destroyed under these conditions, even in an empty bath, so that the contraction is maintained at its full height only for a few minutes. If the Ringer solution be transferred to another

bath with a fresh piece of uterus when the contraction of the first has decreased to half its maximal value, the second piece of muscle only gives half a contraction, so that fatigue is not a very important factor in the falling off of the contraction. If the concentration of adrenalin be maintained by perfusing the bath with Ringer solution containing adrenalin the height of the contraction is maintained.

Effect of some cations Magnesium chloride in a strength of 0.05 p c or less was found to have no appreciable effect on the adrenalin contraction. In a strength of 0.5 p c it slightly diminished the height of the contraction.

The effect on the adrenalin contraction of varying the percentage of calcium in the Ringer solution was studied. In the absence of calcium the spontaneous rhythm of the muscle was diminished. In the presence of an excess (0.5 p c of CaCl_2) the rhythm was increased and after a few adrenalin contractions the muscle went into a state of tone which disappeared when the calcium was washed out again.

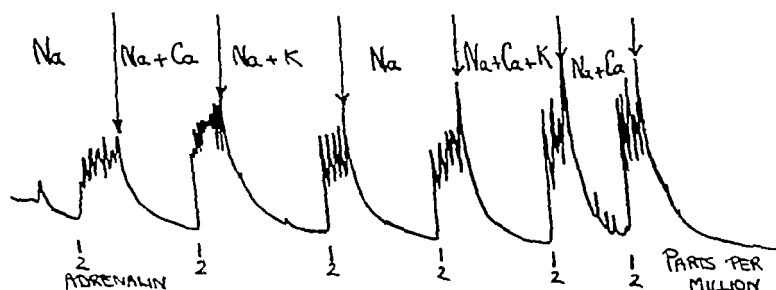


Fig 2 The effect of the presence and absence of K and Ca in the bath. The salts were added to NaCl 0.9 p c. in the following strengths CaCl_2 0.24 p c, KCl 0.42 p c

The absence of both calcium and potassium had little or no effect on the adrenalin contraction—the same heights of contraction were obtained for the same doses. No reversal of the adrenalin contraction was obtained even with small doses of adrenalin as might have been expected from the results of BurrIDGE(4), Wehland(5) and others. A drop of 10 N acetic acid added to the bath reduced the pH to about 5, and greatly diminished the adrenalin response.

The presence of a phosphate buffer (pH = 7.4) had the effect of completely abolishing the spontaneous rhythm without much affecting the adrenalin contraction. Relaxation was delayed. This may be the result of the precipitation of calcium.

The action of ergotamine on the adrenalin contraction

The test for ergot alkaloids is as follows

The reaction of the muscle to adrenalin is tested and then ergotamine is added to the bath, left for 5 minutes and washed out. After 5 minutes in Ringer solution the muscle is again tested with adrenalin to see if it has been paralysed or not. This duration of stay in Ringer is

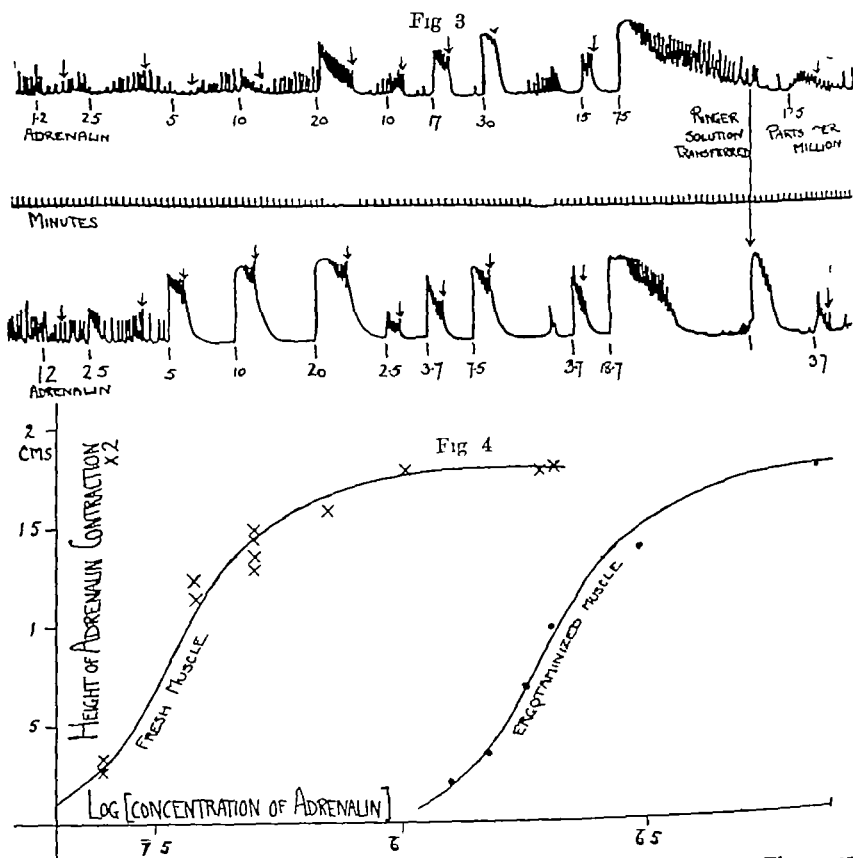


Fig 3 Two pieces of muscle originally giving contractions of similar height. The upper one has been paralysed with ergotamine (five parts per million) for 5 minutes and it now requires four times the concentration of adrenalin to produce contractions of the same size as the lower one. Later the adrenalin was left in the bath until it had practically exhausted its effect on the upper tracing. The Ringer solution was now transferred to the other bath and produced a large contraction of the other muscle.

Fig 4 Curves obtained from the tracing shown in Fig 3 (in the first curve all the heights are reduced in the ratio 2 : 1.8 which was the ratio between the height of maximal contractions when neither piece was paralysed). The curves are drawn exactly the same shape.

essential The paralysis appeared to take 5 minutes or more to develop its full force It was irreversible in that it generally remained constant for at least an hour During this hour, contractions were obtained by giving larger doses of adrenalin Contractions of any height up to the maximum could be produced by increasing the dose of adrenalin in a definite proportion—a proportion which was the same for the different sizes of contraction and was thus, in some sort a measure of the degree of paralysis produced These facts are shown in Figs 3 and 4 It will be seen that the proportion between the doses necessary to produce contractions of the same height, as represented by the distance between the curves (Fig 4), is roughly constant It is a little difficult to get two complete curves from the same portion of uterus, but a number of such curves have been obtained both before and after the action of adrenalin, and they are all of the same general shape If the elements of muscle which are most susceptible to adrenalin were also most susceptible to ergotamine, it might be expected that the small responses to small concentrations of adrenalin would be more affected by ergotamine than the larger ones, because the nerve endings concerned in small contractions would be more paralysed than those concerned in large ones Thus the slope of the curve would be increased. Similarly if there were any correlation between the distribution of susceptibility to the two drugs it would be expected that the shape of the curve would be altered by ergotamine No such alteration was found On the other hand, it was noticed that when uteri were kept overnight they were sometimes sensitive to smaller doses of adrenalin than before and these same uteri were also more sensitive to ergotamine, in that the same concentration produced a higher degree of paralysis It is as if the route to their common site of action had become more permeable This variation in the susceptibility of the whole muscle is thus probably determined by factors different from those which determine the variation among individual fibres, as the former case shows correlation between the susceptibility to the two drugs, and the latter does not

When similar pieces of the same uterus were tested with varying concentrations of ergotamine, it was found that over as wide a range as was convenient the adrenalin proportion bore a linear relation to the concentration of ergotamine

Similar results were obtained by Langley(5) in his study of the effect of curari and nicotine on the tone of the rectus abdominis of a frog, and by Cushny(6) with pilocarpine and atropine Cushny points out that the results do not fit in with the conception of a simple chemical

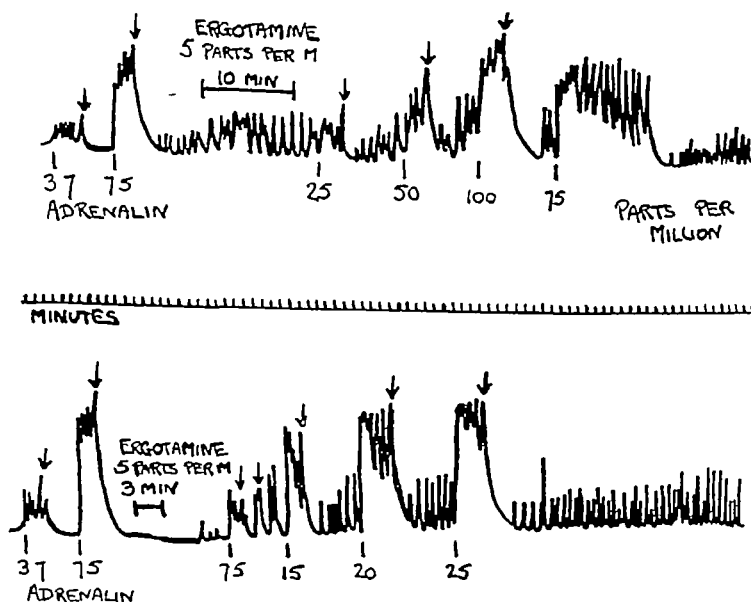


Fig 5 Tracings showing the paralysis when ergotamine was left in the bath for different times. In the upper tracing the response to 50 parts per million of adrenalin is intermediate between that to 37 and that to 75. Thus the adrenalin proportion lies between $50/37$ and $50/75$ or 1.32 and 0.66 . It is probably about 1.0 because the response to 75 is equal to that to 75 . Similar reasoning applies to the bottom tracing where the proportion lies between 2 and 4 and is probably about 3 .

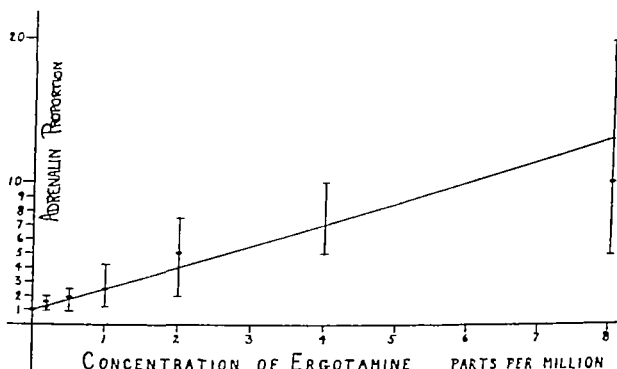


Fig 6 Adrenalin proportion when different concentrations of ergotamine were left in the bath for 5 minutes. The vertical lines show the limits between which one can be certain of the proportions. (See discussion under Fig 5)

combination of the two drugs—leaving a certain amount over. In confirmation of this it was not found possible to detect any action between ergotamine and adrenalin *in vitro*. A mixture of solutions of the two, incubated overnight, produced an effect on the uterus indistinguishable from that produced by the same solutions introduced independently into the bath—an adrenalin contraction falling rapidly after 3 or 4 minutes as the ergotamine came into action.

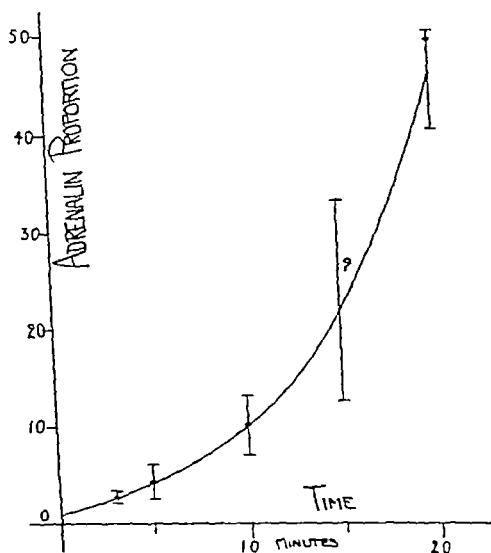


Fig 7 Adrenalin proportion when five parts per million of ergotamine were left in the bath for different times

The same dose of ergotamine was left in the bath for varying times and the adrenalin proportion determined. In all cases it was found that the degree of paralysis increased with time. The relation is shown in Fig 7.

In some experiments with smaller concentrations of ergotamine there were indications that the paralysis reached a maximum value in from 3 to 20 minutes, but it was found impossible to get precise information as to the shape of the curves. When the ergotamine was left in the bath for a long time, the muscle developed additional tone which obscured all further results. It is possible by taking any one value of the adrenalin proportion and reading off in Fig 8 the corresponding values of the concentration of ergotamine and the time it has had to act to get some idea of the relation between these two quantities.

The series of such curves obtained by taking different values of the adrenalin proportion are similar in general shape to those published by Sollman(7)

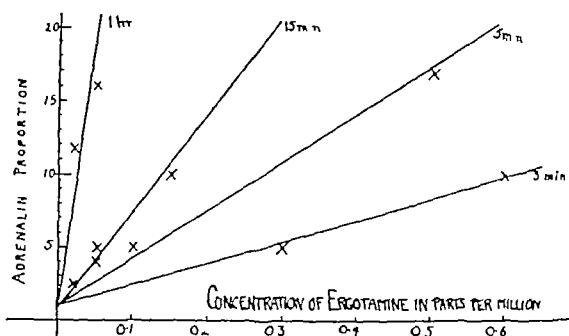


Fig 8 Effect of varying both the concentration of ergotamine and the time it was left in the bath with different parts of the same uterus

While the present paper has been in preparation Braun(8) has published a study of the action of adrenalin and ergot alkaloids on rabbit's uterus in which he makes the statement that the product of the concentration of ergot necessary to produce a given effect and the time it is left in the bath is constant. This would give a rectangular hyperbola as the curve connecting the two quantities. It is quite possible that Sollmann's curves and mine are rectangular hyperbolæ.

An attempt was made to demonstrate the disappearance of ergotamine from the bath by allowing it to act on one portion of muscle and then transferring the Ringer to another bath in which was a fresh bit of muscle. The second piece was paralysed to the same extent as the first, no measurable quantity of ergotamine having disappeared.

Thus the amount of ergotamine passing into the muscle is dependent on (1) The concentration of ergotamine in the bath (2) The time the muscle is in the ergotamine. Ergotamine appears to continue to pass in for over 20 minutes in some cases (3) The temperature. Two corresponding halves were tested with adrenalin and then placed in ergotamine for 5 minutes—one at 37° and the other at 5°-2°. The muscles were then replaced in their baths and re-tested. The former was more paralysed than the latter.

It is independent of (1) The bubbling of oxygen. Two corresponding halves were tested with adrenalin and then paralysed by being left in contact with ergotamine for 15 minutes. In one the oxygen was left bubbling all the time and in the other it was turned off after 2 minutes.

when mixing was probably complete. The degree of paralysis produced in the two cases was identical and corresponded with other values obtained for the same uterus. (2) The presence of adrenalin in the bath

Ergotamine was not concentrated in the muscle sufficiently to produce a measurable alteration in the concentration of ergotamine in the bath.

After passing in, the ergotamine reacts in some way with the tissues. This reaction is not complete when the muscle is removed from the ergotamine. Its rate has a positive temperature coefficient.

Two corresponding halves were paralysed in the bath for 3 minutes and then immediately placed in Ringer solution for 12 minutes—one at 37° and the other at 2°. They were then replaced in the bath in fresh Ringer solution and retested 4 minutes later. The former gave a smaller contraction with adrenalin than the latter. After 10 minutes' further rest, the second stage was complete in both and they both gave the same result.

It was thought possible that this reaction might consist in the adsorption of ergotamine on some structure in the muscle. The evidence of the above experiment does not of course bear on this point, as adsorption, in common with most other processes, proceeds more quickly at higher temperatures. The following experiment is more relevant.

In a piece of muscle in which both stages had been completed in the bath, an attempt was made to change the equilibrium point by placing the muscle for 15 minutes in cold Ringer solution 2° and then replacing and retesting it. The degree of paralysis might have been expected to alter by about 20 p. c. under these conditions but no change could be detected. But of course it is very hard to say, what would or would not be the properties of such an adsorption and it is not possible to lay great stress on the result of this experiment.

A rough measure of the ergotamine content of a sample of ergot may be obtained with only two pieces of uterus by measuring the adrenalin proportions in the two cases. No improvement on Broom and Clark's technique for the final readings is suggested. The time must be accurately measured. If times longer than five minutes be used, the sensitivity of the test is increased but the percentage accuracy is unchanged.

Theory of mode of action. A theory, which cannot be fully discussed here, has been formed on the following lines. It is assumed that there is an area in the muscle on which the adrenalin must act and that a fraction of this area is blocked by ergotamine so that in any given case the concentration of adrenalin must be increased in a certain proportion to produce the same effect. If it be assumed as was found experimentally, that this proportion bears a linear relation to the concentration of ergotamine in the bath, the relation of this latter variable to the degree of action of the ergotamine may be calculated. The curve is of form similar to that found for the same relation in the case of adrenalin.

SUMMARY

1 The effect of ergotamine and adrenalin on rabbit's uterus suspended in a bath has been studied The relation of the concentration of adrenalin to the height of contraction has been measured

2 If ergotamine be added it may take more than 20 minutes to develop its complete effect This appears to take place in two stages—the first is a simple diffusion into the muscle The second stage is some further change within the muscle

3 Its effect is to increase in a certain proportion the concentration of adrenalin necessary to produce a contraction of given height

4 Its degree in terms of this proportion bears a nearly linear relation to the concentration of ergotamine

5 The effect of different temperatures on this paralysis has been studied Both stages proceed more quickly at 37° than at 0°

6 The simplest theory that can be suggested to account for these results entails a number of elements of varying threshold to adrenalin and of varying accessibility to ergotamine

I wish to thank J W Trevan for his friendly criticism and advice

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THE IMPULSES PRODUCED BY SENSORY NERVE-
ENDINGS Part 2 The response of a Single End-Organ
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IN Part I of the present series⁽¹⁾ one of us described an instrument consisting of a capillary electrometer with a 3-valve amplifier, capable of recording the action currents set up in sensory nerve fibres by appropriate stimulation of their end-organs. A preliminary analysis of the results was given, but it was pointed out that an essential step was missing. All the records were made from nerve trunks containing many afferent fibres and it was impossible to tell with certainty what was happening in each fibre, what was the frequency of the response, how it varied with the stimulus, etc. The present paper remedies this defect, for it is concerned with the impulses set up by a single end-organ and travelling in a single afferent nerve fibre. Only one type of end-organ has been investigated, but the results seem to be of such general application that it will be surprising if other types are found to behave very differently.

Preparation employed. The recording instrument has already been described in detail and the only modification introduced has been the provision of a variable shunt to reduce the excursions of the electrometer, should this be necessary. The preparation employed was suggested to us by the papers of Keith Lucas on the all-or-none response of muscle fibre. In these experiments Lucas used the m. cutaneus dorsii of the frog. He found that the nerve supplying this muscle had ten fibres only, of which one or two might be sensory. We have investigated this muscle, but so far we have been unable to detect any action currents in the nerve roots when the muscle is stretched, and we are inclined to think that all the fibres are motor. But in a footnote Lucas mentions another muscle—the sterno-cutaneous—in which there is no doubt of the existence of sensory end-organs. The number of fibres in the nerve supplying the muscle varies from 12 to 25. There is certainly one muscle-spindle in the muscle (it is figured in Cajal's book, *Textura del Sistema Nervioso*, Madrid, 1899, p. 404), and as our results show there are usually three or

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four end-organs which are stimulated by tension in the muscle of a medium-sized frog (*R temporaria*). We have used this muscle in all the experiments to be described. The preparation is fairly simple. The skin on the chest of the frog is removed except for a small piece in which the cephalic end of the muscle is inserted. This piece of skin serves as the point of attachment of the silk thread by which the muscle is stretched. The origin of the muscle is not interfered with but the fascial investments on either side are cut through. The nerve is derived from a branch of the large brachial nerve, and in dissecting it the brachial nerve is cut high up in the axillary space and again just below the branch which supplies the sterno-cutaneous. This is followed down from the brachial nerve, all side branches being cut, until it becomes reduced to the extremely slender trunk which enters the muscle about half-way down its outer border. The preparation is tested by stimulating the nerve with an induction shock. This should produce a contraction in the sterno-cutaneous and in no other muscle, and as a rule there is no difficulty in securing this condition. The preparation is placed inside the metal box which acts as a shield from electromagnetic disturbances (see Part I), with the nerve slung by a thread from a small wooden peg at the side of the frog. Non-polarisable electrodes of the Ag, AgCl, NaCl, gelatine type are connected to the nerve by short lengths of moist carpet thread embedded in the gelatine. These allow some movement of the nerve to take place (and this is bound to happen when the muscle is extended) without producing changes of potential in the electrometer. For stimulating the end-organs in the muscle we have used weights of $\frac{1}{2}$, $\frac{1}{4}$, 1, 2, 3 and 5 grm attached to the thread from the muscle and allowed to hang over a light pulley. The maximum tension developed by the sterno-cutaneous of a medium-sized frog in an isometric contraction produced by tetanising the nerve was 2 grm so that the above range of tensions probably covers the values occurring naturally in the intact animal. In some of the experiments we have used an elastic apparatus which permits of gradual extension. This will be described later. The records of the action current were made with 3-valve amplification (= 1850) on plates travelling at 1 metre per sec or less and on cinematograph film travelling 10–15 cm per sec. Since the nerve trunk (*i.e.* the nervus pectoralis proprius, Gaupp) to which the electrodes are applied contains only 50 to 200 fibres, the responses are large and they are sometimes so large that their size must be reduced by shunting the electrometer or by bringing the leads very close together on the nerve, so that the two phases of the response interfere. The latter method is usually preferable,

since a change in the resistance of the electrometer circuit alters the constant used in the analysis of the records

Results In our earliest experiments we recorded the responses in the nerve with various weights which were hung on the muscle 10 seconds before the plate was exposed and remained on it during the exposure. The interval of 10 seconds between the moment of application of the weight and the making of the record was chosen to allow the conditions to become steady before the record was made. The records all showed a succession of action currents with the same form and time relations as those found in the frog's sciatic (cf Part I), occurring in irregular succession at frequencies which were as high as 150 per second with a 2 gm weight. Most of the action currents conformed to a standard size, but some were larger, and on analysis many of these proved to be made up of two or more responses, separated by a very short time interval. The interval between these responses was often as short as $1/1000$ sec or less, and this interval is shorter than the absolute refractory period of the frog's sciatic (about 0.02 sec 15°C). It was therefore obvious that the responses were not all produced by the same nerve fibre, and we attempted to diminish the number of end-organs or nerve fibres in action by cutting narrow parallel strips away from the muscle on the mesial side. As a rule the first section produced a considerable reduction in the frequency of the responses for a given stimulus and evidence of regular rhythms began to appear in the records. As more and more of the muscle was cut away the existence of definite rhythms became more and more obvious. The change that occurs is best illustrated by the records from our most successful experiment of this type, Exp 1 (Fig 1). This was made from a very large male frog and the muscle and its nerve were isolated from the body, the abdominal muscles from which the sterno-cutaneous takes its attachment being held in a clamp. In *A* the muscle is intact and the load is 2 gm. In *B* the stimulus is the same but the first strip of muscle has been removed and the responses are fewer and more regular. In *C* a second cut has been made and the stimulus was reduced to 1 gm. to avoid damaging the muscle. An analysis of this record shows that the responses occur in four regular series with periods of 0.35, 0.42, 0.45 and 0.46 sec. After the next cut the responses become completely regular with a period of 0.30 sec. At this stage of the experiment about two-thirds of the muscle had been removed and the subsequent removal of another small strip abolished the responses completely, though what remained of the muscle would still contract when the nerve was stimulated.

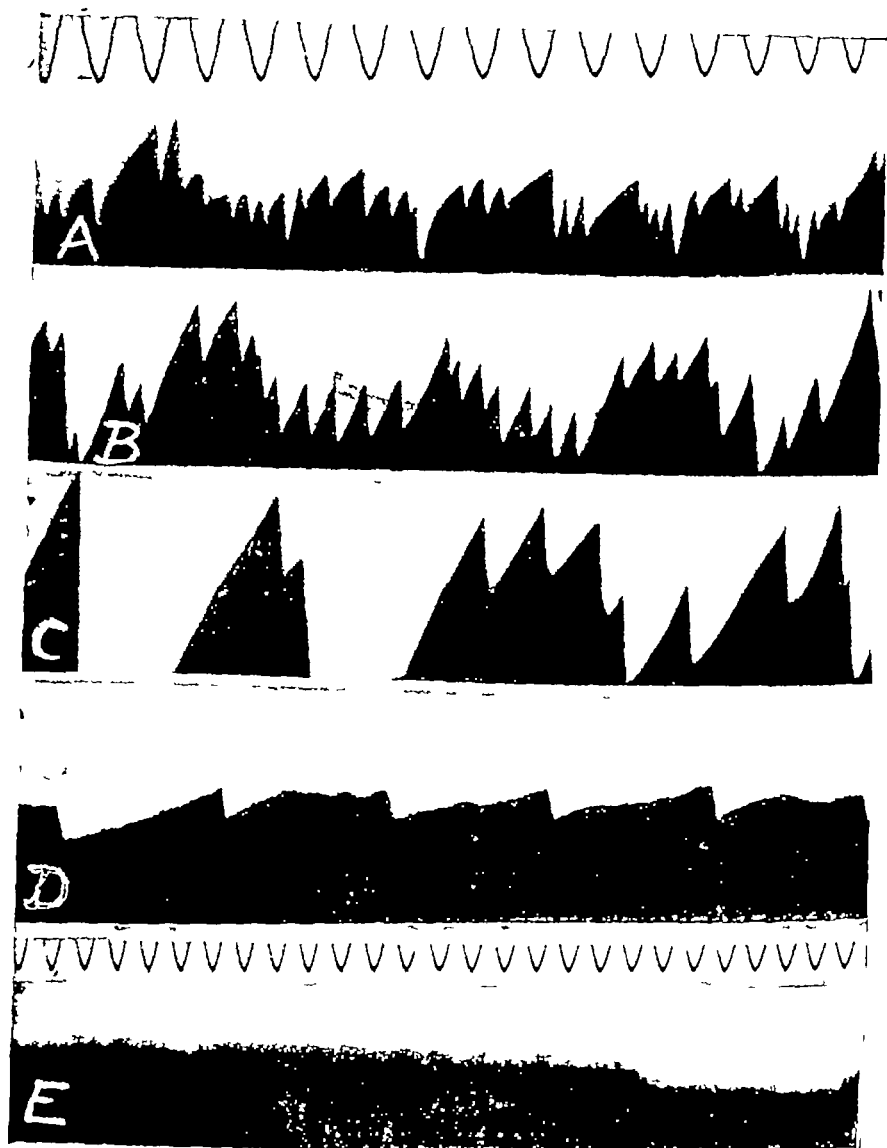


Fig 1 Exp 1, 19° C Afferent responses from nerve when muscle is stretched by a weight applied 10 sec. before record is made Capillary electrometer with 3 valve amplifier, magnification 490 Time marker gives 0.1 sec Responses vary in size, as electrodes are adjusted between each record.

- A Muscle intact 2 gram weight
- B First strip removed. 2 gram weight
- C Second strip removed. 1 gram weight. Impulses in four regular series.
- D Third strip removed. 1 gram weight. Single regular series
- E Fourth strip removed. 1 gram weight. Slower plate. No impulses.

Response from single end-organs We have no direct proof that the regular responses shown in *D* are due to one nerve ending only, but the indirect evidence leaves very little doubt of it. As many of our conclusions are based on this assumption, the evidence needs consideration in some detail. In the first place the impulses recorded in Fig 1 *A-D*

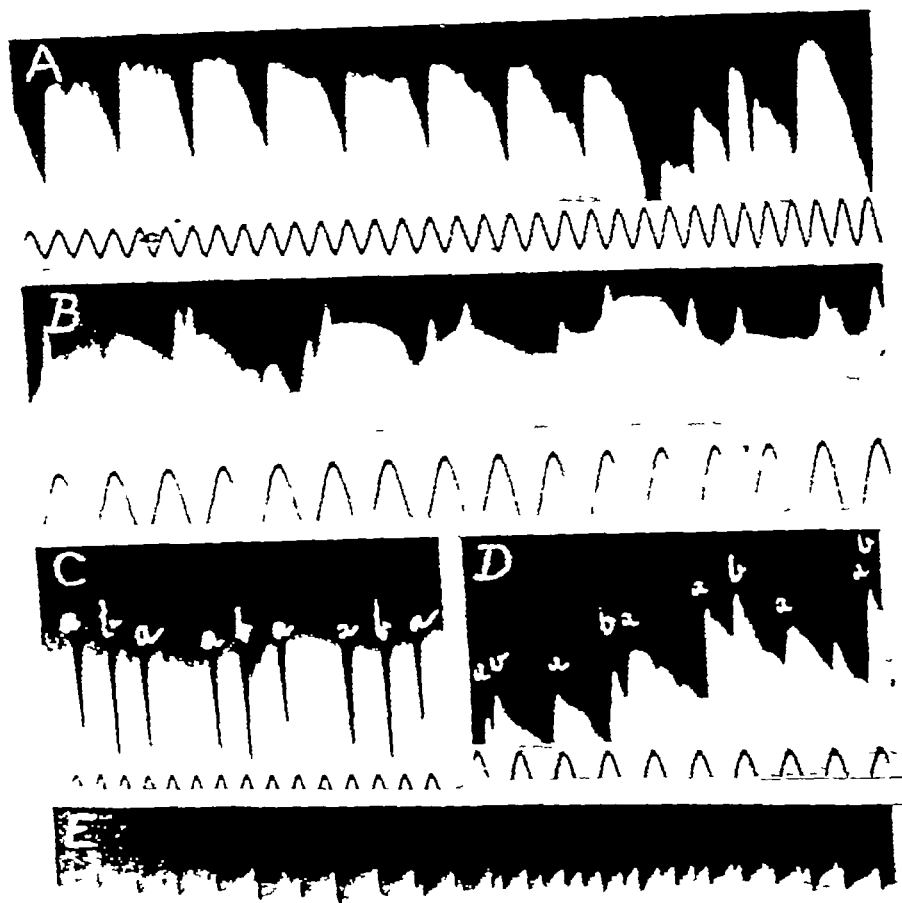


Fig. 2. Examples of rhythmic discharge from different experiments

- A* Exp 5 15° C Muscle divided. 3 grm., 10 sec. Single rhythm, broken at end.
- B* Exp. 2 21° C Muscle divided. 3 grm. 10 sec Double rhythm.
- C* Exp 5 15° C Muscle divided. 2 grm. 10 sec Double rhythm.
- D* Exp. 6 15° C Muscle divided. 2 grm., 10 sec. Double rhythm, one extra.
- E* Exp 15 14° C Film. speed as in Fig 5 *D* Loading just complete. Two rhythms producing beats

are clearly set up in sensory and not in motor fibres. If traction on the muscle produced impulses in the motor nerve fibres, there is no reason why the removal of little more than two-thirds of the muscle should abolish the responses, for the effects of stimulation showed that the motor nerve supply to the remaining muscle fibres was still intact. When several end-organs are present, as in the intact muscle, there is no indication that they respond synchronously, for the impulses in *A* occur at irregular intervals, and no reason why they should so respond to a stimulus which is continuous and not periodic. Yet in *D* the responses occur so regularly that it is quite impossible that they should be produced by two or more end-organs acting independently. The conclusion must be that these responses are the product of a single end-organ. The histological evidence is certainly not opposed to this conclusion for the preparation used in *D* cannot have contained more than ten intact nerve fibres at an outside estimate, and of these the majority are clearly motor, as may be seen from stained specimens.

With this preparation the rhythm of the responses remained perfectly regular except with the weakest stimulus ($\frac{1}{4}$ grm weight) where the rhythm was occasionally broken. In at least three other preparations made in the same way, we have obtained records showing a single, regular rhythm with certain strengths of stimulus, but with stronger stimuli, or in other records with the same stimulus, either a few isolated extra responses have appeared or else the record is made up of two or more distinct series of responses side by side. Fig 2 gives examples of such records. Double rhythms are shown very clearly in *B*, *C*, *D* and *E*, though in *B* and *C* there were one or two responses which do not fit into the scheme. These aberrant responses will be dealt with later. For the present we may conclude that as more and more of the muscle is cut away the frequency of the afferent responses produced by stretching is reduced, a stage is reached in which they can be analysed into two or three regular series and a final stage in which only one regular series is left. Each regular series we take to be the product of a single end-organ.

Production of regular discharges. In these experiments the stimulus, i.e. the change of conditions imposed on the muscle, is steady and has no period of its own. To make certain of this we have altered the length of the thread from the muscle to the weight and allowed it to run over a small cardboard bridge placed at various distances from the muscle. These procedures would alter the period of any possible vibrations in the thread, but they have no effect on the rhythm of the response. But it

is not surprising that an end-organ should produce a regular series of discharges under a steady stimulation it would have been much more so had the discharge been irregular. Indeed, the regular response of the end-organ can be very simply explained without recourse to any other factors than the known properties of excitable tissues in general. In a nerve or muscle fibre a momentary stimulus sets up an impulse which leaves the refractory state behind it. During the absolute refractory period a second stimulus has no effect, but as the refractory state passes away the excitability of the nerve returns gradually. A second stimulus falling during this relative refractory period will succeed in exciting if it is strong enough, and as recovery progresses the strength of the stimulus may be reduced until it is finally no stronger than the threshold value for the resting nerve. The course of events is shown by the "recovery curve" of which many examples have been previously published. The curve in Fig 3 A is a typical example from a frog's sciatic at 15° C.

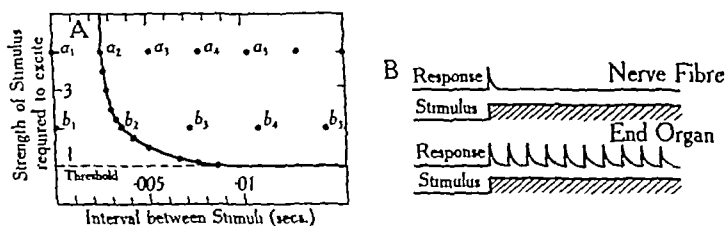


Fig 3 A Recovery curve of frog's sciatic at 15° C

B Response of nerve fibre and of end organ to a constant stimulus

Now if a constant current is used instead of an induction shock, an impulse is set up when the current is turned on, but no further impulses are produced till it is broken again (unless the strength of the current is very great). The impulse leaves the usual refractory state which is recovered from in the usual way, but the constant current does not re-excite, because the nerve has now become adapted to it and it is no longer an effective stimulus. Various hypotheses have been put forward by Nernst, Hill, Lapicque, etc. to account for this rapid adaptation, but these need not concern us for the moment. The adaptation is also shown by using a current which increases gradually instead of suddenly. Unless the rate of increase exceeds a certain "liminal current gradient" (Lucas) no impulse is set up.

In most, if not all, sensory end-organs this process of adaptation, if it occurs at all, is evidently a much slower affair. A continued stimulus, such as a state of tension, a light or a sound, produces a continued

passage of sensory impulses to the central nervous system. The sensation diminishes after a time, but it does so very slowly. The difference between the response of a nerve and an end-organ to a constant stimulus can therefore be represented diagrammatically as in Fig 3 *B*. Now if we assume that the end-organ reacts in exactly the same way as the nerve fibre, with this one exception of a much slower rate of adaptation, it is obvious that a steady stimulus will produce a regular discharge of impulses. When the stimulus is first applied an impulse will be set up and the end-organ will become refractory. It will recover and, as soon as its excitability had returned to the requisite value, another impulse will be set up. If the stimulus has the strength α_1 (Fig 3 *A*) the impulses

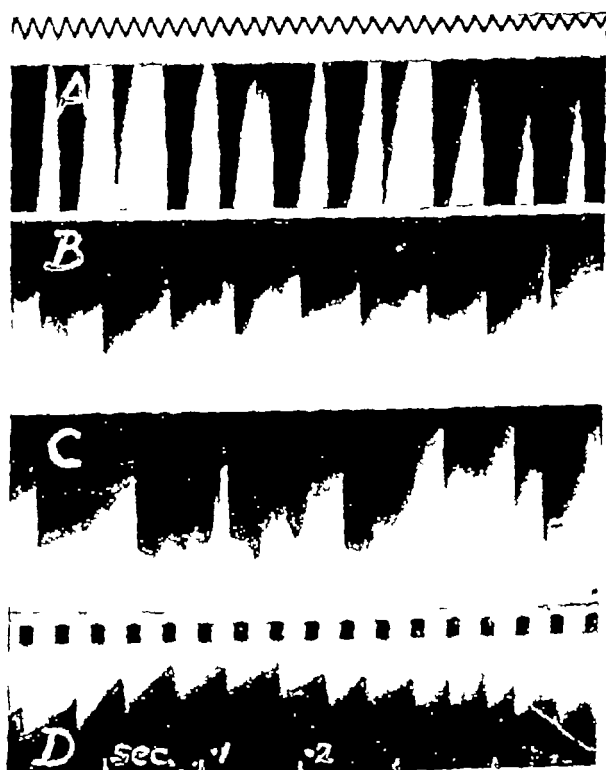


Fig 4 *A*, *B* and *C* Exp 1 Single end organ in action. Rhythm with various loads.

A 1 gram, 10 sec Frequency 33 per sec.

B $\frac{1}{2}$ gram, 10 sec Frequency 27 per sec

C $\frac{1}{4}$ gram, 10 sec Frequency 21 per sec Less regular

D Exp 15 Cinematograph film Gradual loading and progressive increase in frequency

will recur at a_1, a_2, a_3, a_4 , etc. If it is weaker, b_1 , the impulses will recur with a slower period b_1, b_2, b_3 , etc. If it increases gradually in strength, the frequency of the impulses should increase too.

The end-organs of the sterno-cutaneous behave as we should expect on this hypothesis. Fig 4 *A, B, C* shows three sets of responses from Exp 1 with different weights applied for 10 secs. The weights were 1 gm, $\frac{1}{2}$ gm and $\frac{1}{4}$ gm and the corresponding periods are 0.30, 0.37 and 0.46 sec. The records were made at intervals of 1-2 mins. The responses in *A* (1 gm) are very large and they were reduced in the subsequent records by moving the electrodes closer together. With the $\frac{1}{4}$ gm weight there are signs of some irregularity in the rhythm, but the dominant rhythm is clearly present. Fig 4 *D* shows the effect of a gradual increase in the stimulus. In this experiment the weight was lowered very slowly on to the string from the muscle by the movement of a weighed lever controlled by an oil dashpot. There is a gradual increase in frequency and later on a second rhythm begins to appear as the stimulus becomes adequate for a second end-organ.

Recovery curve of end-organ By plotting the intervals between consecutive responses against the strength of the stimulus we shall evidently obtain a "recovery curve" for the end-organ comparable to the recovery curve of a nerve fibre. A curve constructed in this way for Exp 1 is given in Fig 5 *A*. The threshold stimulus—the least tension which will evoke any response—is unknown, so that the strengths of stimuli must be expressed in absolute values and not as multiples of the threshold strength. Similar curves, or portions of curves, from some other experiments, are given in the figure. In some of these the larger weights brought several nerve fibres into action, so that there is some uncertainty in the upper parts of the curve. The general form of the curve apart from its actual time relations can be studied most easily by counting the frequency of the responses from an intact muscle with three or four end-organs, instead of waiting for the lucky chance of a preparation with only one. The average interval between consecutive responses plotted against the strength of the stimulus will give a curve with time relations three or four times as rapid as those of the single organ. The only objection to this method is that a strong stimulus may call into play more end-organs than a weak, and consequently the intervals with strong stimuli will be unduly shortened and the curve will rise more gradually than it would have done if the number of organs in action had been constant. Fig 5 *B* shows the relation between frequency and weight in an experiment with the muscle intact. The figures opposite each point give the

order in which the records were made. Fig 5 C is the recovery curve constructed from this and the dotted line is the hypothetical curve for

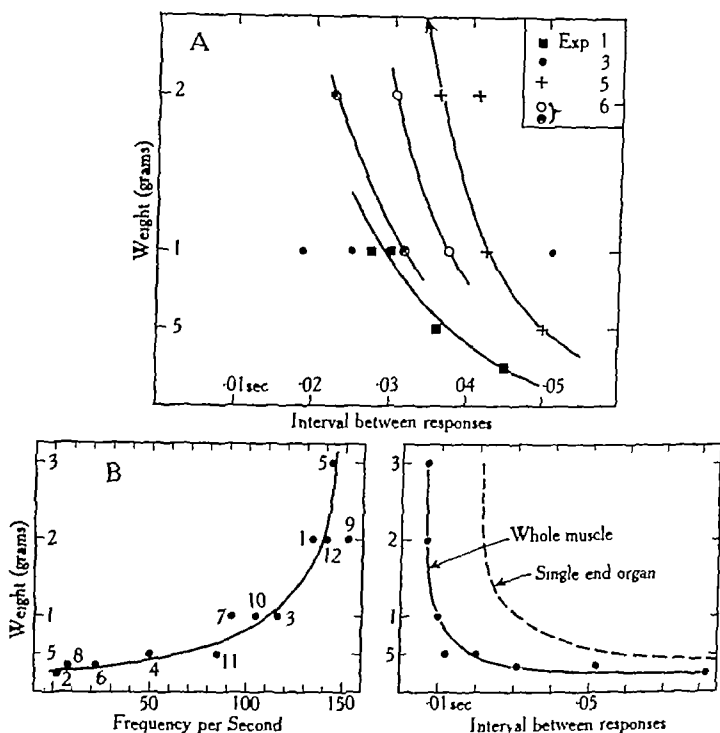


Fig 5 A Relation between stimulus and period of rhythmic responses in different experiments

B Exp 9 17° C Relation between stimulus and frequency of response, muscle intact

C Exp 9 17° C Relation between stimulus and interval between responses for intact muscle. Hypothetical curve for single end organ

a single end-organ made on the assumption that three are always in action. Although the curves for single end-organs have to be constructed from very meagre data their general features agree fairly well. With a stimulus of 5 gm the periods are over 0.3 sec, and with one of 2 gm they lie between 0.3 and 0.2 sec. An increase to 3 gm and above reduces the period slightly, but the most rapid period which has appeared is 0.15 sec.

The time relations of the recovery curve for the end-organ are therefore considerably longer than those for the nerve fibre. In a mixed nerve trunk such as the frog's sciatic, the absolute refractory period, judged by the presence or absence of a second electric response is rarely longer than 0.025 sec at 15° C. The return of excitability has only been

mapped out for the motor fibres, but it is unlikely that it differs much from that of the sensory fibres from the muscle. Erlanger and Gasser(2) have shown that a mixed nerve trunk may contain fibres whose refractory periods may vary from 0.0142 to 0.0446 sec but the phrenic nerve of the dog contains motor fibres and sensory fibres from muscle receptors and they find that all these have the same refractory period. For the motor fibres of the frog the excitability has returned to 95 p.c. of its resting value in less than 0.2 sec though the subsequent return to complete resting excitability may be complicated by the development of the super-normal phase. Thus, if we may assume that sensory and motor fibres recover at much the same rate, it appears that the relative and absolute refractory periods last much longer in the end-organ than they do in the nerve fibre connected with it. In fact, the periods in the end-organ and the nerve fibre seem to be so related that the impulses set up will always travel in nerve which has completely recovered from the passage of the previous impulse.

Effects of adaptation. Before this conclusion can be accepted there is another factor which must be taken into account. It was pointed out in Part I that the frequency of the impulses in the sciatic when the gastrocnemius is stretched falls off considerably as the period of stimulation is increased. There is evidently some adaptation of the end-organ to the stimulus, as indeed we should naturally expect. This is true for the single end-organ as well as for the collection of end-organs in the

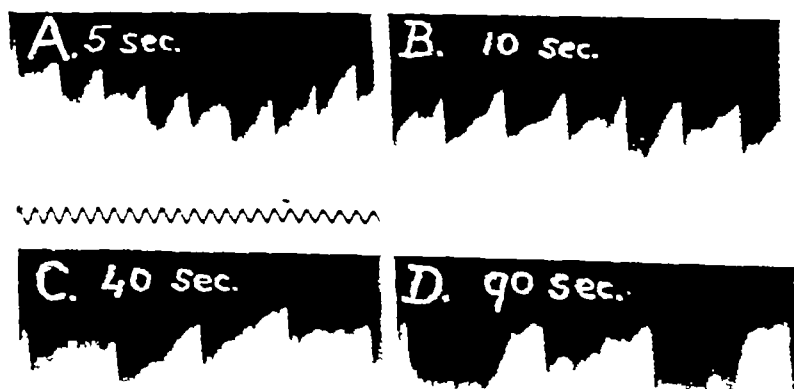


Fig 6 Exp 1 Single end organ. Decrease in frequency of response as duration of stimulus is increased. 1 grm. weight

gastrocnemius Fig 6 gives the responses in Exp 1 produced by a 1 gm weight applied for 5, 10, 40 and 90 seconds and the intervals between the responses range from 0.26 sec when the weight had been on for 5 sec to 0.8 sec when it had been on 90

Consequently the recovery curves given in Fig 5 are only true for an end-organ which has already been stimulated for 10 seconds, and they are only true for this period on the assumption that the rate of adaptation is the same for a strong stimulus as for a weak. We have, therefore, studied the process of adaptation in greater detail with the aid of continuous records on long strips of cinematograph film. In the earlier experiments the weight was lowered by hand so that the tension was applied suddenly. A strip of film was exposed before the loading and another beginning about 1 second afterwards and continuing for 20 seconds or more whilst the weight was in place. At the actual moment of loading there was usually a large excursion due apparently to the movement of the nerve and the impulses were not recorded until this had subsided. An experiment of this kind is shown in Fig 7. The muscle was intact and the frequencies are reckoned by counting the total number of impulses in each second. With a 3 gm weight the frequency at the end of 1 second is 145 and at the end of 10 it is 104, *i.e.* it has fallen to 72 p.c. of its former value. With a weight of 5 gm the frequencies are 58 and 40, *i.e.* a fall to 69 p.c. In another experiment the weight was lowered by the movement of an arm controlled by an oil dashpot. With this method the extension of the muscle occupied about 5 sec and was smooth enough to allow the record to be made during its progress. The data below give the maximal frequency just after loading and the frequency 10 seconds later with different weights.

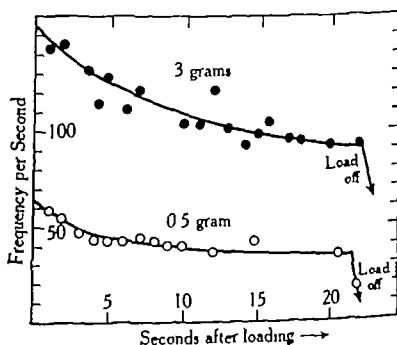


Fig 7 Exp 11 Decline in frequency after application of load

Exp 12 Temp 14° C

Weight gm	Initial frequency (maximal) per sec	Frequency after 10 sec loading per sec	Percentage of initial frequency
5	190	120	63
5	150	104	69
5	35	15	43
1	66	37.5	57
2	98	59	60

In this experiment some of the waves were monophasic and some diphasic. These can be distinguished when the frequency is not too high and the rate of adaptation can be checked by counting the monophasic waves only. This gives the following results

Exp 12 Monophasic waves only counted.

Weight gram.	Initial frequency (maximal) per sec.	Frequency after 10 sec loading per sec.	Percentage of initial frequency
2	37	17	45
1	26	16	61.5
5	14	8	57

Later experiments using an elastic extension apparatus instead of a weight gave results of the same order and the agreement between them is close enough to justify the conclusion that the frequency after 10 seconds of weighing is from 40 to 70 p c of the initial frequency and that the percentage fall is much the same whatever the value of the stimulus.

Thus the recovery curves in Fig 5 are approximately correct for an end-organ which has been in action for 10 seconds and we can obtain the curve for the fresh, unadapted state by halving the time intervals for each point in the curve. This has been done in Fig 8, taking the recovery

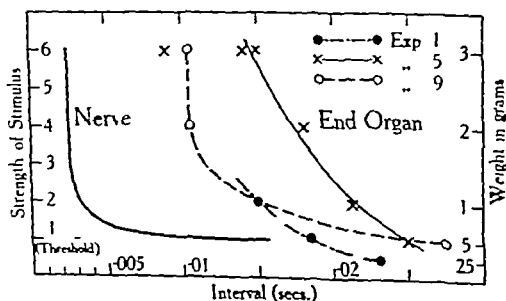


fig 8 Recovery curve of frog's sciatic at 15° C and corrected recovery curves for end organ at moment of application of load.

curve in Fig 6 A and C as a basis, and in the same figure is drawn a typical recovery curve of the motor fibres in the frog's sciatic. Evidently the rate of recovery of the end-organ is so much slower than that of the nerve that the impulses set up by it will always travel in a fibre which has itself almost completely recovered. This conclusion is interesting, for it shows that, on the afferent side at least, the refractory period of the nerve fibre is not one of the factors which determines the size or

frequency of the impulses which reach the central nervous system. Owing to its very rapid recovery the nerve fibre is able to behave alike to all the impulses which reach it from the end-organ. It should be able to conduct all at the same rate and with the same intensity because the path can never become overcrowded by too frequent discharges. Cooper and Adrian (2) have already brought forward some evidence to show that the frequency of impulses reaching the motor neurones is also lower than the maximum frequency to which the motor fibre and the muscle can respond, so that both on the motor and sensory side it seems that the nerve fibres are never pushed to the limit of their power of response.

All-or-nothing response of nerve fibre As far as the present experiments are concerned there is an interesting corollary to this conclusion. If the afferent impulses are all travelling in nerve fibre which has completely recovered, they should all be of the same size provided that the all-or-nothing principle does apply to the nerve fibre. Since the potential difference developed varies with the degree of moisture on the nerve and the distance between the electrodes, the point must be tested by comparing records in which both these factors are kept constant. In the record shown in Fig. 4 D the stimulus is increased gradually, the state of the nerve and electrodes remaining unchanged. It will be seen that the impulses increase in frequency but that the rate of rise of the mercury at each response shows no appreciable change. Records of this kind are unsuitable for analysis, since they are taken on a slowly moving surface, and to test the point more rigorously we have taken a series of responses with different weights on a plate travelling at 1 metre per sec. The weights were hung on for 10 seconds before the exposure was made and records were made as rapidly as possible so as to avoid changes due to the drying of the nerve. The muscle was a large one, containing probably five or more end-organs and half of it was cut away before the records were made. In this state it contained at least two end-organs but the responses were far enough apart for analysis. The data of this experiment are shown below.

Exp 10 Temp 14° C

A. Muscle intact Frequency of impulses with various loads determined at the moment of loading

Weight gm	Frequency per sec
25	137, 175, 162
5	200
10	290
20	400

Exp 10 Temperature 14° C (contd)

B Muscle cut Loading for 10 seconds Size of responses from analysis of electrometer records

Order of exposure	Weight gram.	Maximum height of response (arbitrary units)			
1	25	35,	40		
2	20	35,	37		
3	10	34,	37,	34	
4	25	34.5,	29,	39	
5	5	37,	35		
6	20	35,	39,	41	
7	25	34,	38		
8	10	30	35,	35,	33
<hr/>					
Averages	25	35.6			
	5	36			
	10	33.7			
	20	37.4			

It will be seen that an eight-fold increase in the stimulus gives no appreciable increase in the size of the response. That the $\frac{1}{4}$ gm stimulus was not already maximal is shown by the increase in frequency with the larger weights. The experiment seems conclusive enough and a general survey of all our records has detected no sign of an increase in the size of each response when the stimulus is larger.

It is conceivable that the electric response may show no variation although the change which evokes it—the propagated disturbance—does vary, but there is at any rate no doubt that there is an all-or-nothing relation between the stimulus applied to the end-organ and the electric response in the afferent nerve fibre. This result confirms the conclusions already put forward by Adrian and Forbes(4) and gives evidence of a new kind in favour of the all-or-nothing reaction of the nerve fibre.

Grading of effect on central nervous system. To reconcile an all-or-nothing relation in the sensory nerve fibre with the obvious fact that our sensations are graded, it has often been pointed out that the effect on the central nervous system may be determined by two other factors apart from the size of each impulse. These factors are the number of fibres in action and the frequency of the responses in each fibre. Since our preparations contained relatively few end-organs even with the muscle intact, it was nearly always possible to count the total number of impulses per second with stimuli of different strengths.

Some curves relating the total frequency to the strength of the stimulus are given in Fig 9. In all of these the weight had been on for 10 seconds before the record was made. On the average the frequency rises from about 20 per second with a $\frac{1}{4}$ gm weight to 200 with 5 gm., but there is considerable variation from one muscle to another. This is

probably due to differences in the number of end-organs, for the muscles of large frogs usually gave the higher frequencies. The form of the curves

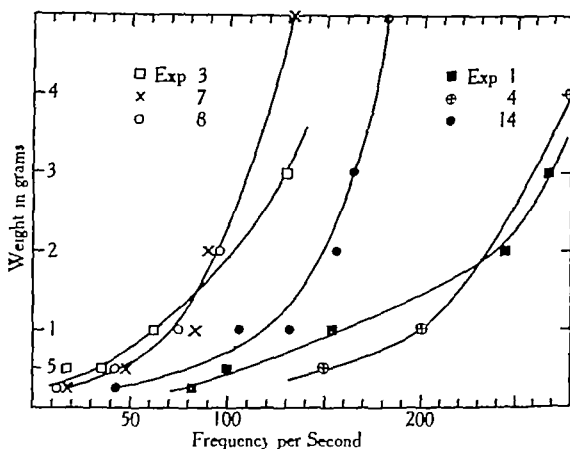


Fig 9 Relation between frequency and strength of stimulus for various intact muscles.

suggests that most of the end-organs are brought into play by a stimulus of 1 or 2 grm since there is not much increase in frequency with larger weights. Evidently a much wider range of gradation would be possible in a muscle with many end-organs of varying excitability, and the form of the curve would then depend more on the distribution of thresholds in the different end-organs than on the change of frequency in each fibre. For this reason no general conclusion can be drawn as to the precise relation between the stimulus to a group of end-organs and the total frequency of impulses which will reach the central nervous system.

The smallest stimulus which we have been able to use was the weight of $\frac{1}{4}$ grm. Smaller weights were not great enough to produce any constant degree of extension owing to the friction of the thread and the muscle on the moist surface of the chest. But in the majority of our experiments a slow discharge of impulses continues (as long as some end-organs are present) even though the muscle is perfectly slack. These impulses occur at frequencies ranging from about 3 to 8 per second. The interval between successive impulses often remains constant for half a second or more, but it is hard to trace a definite rhythm over longer periods. An example of these responses is given in Fig 11. It is conceivable that they may be the basis of the slight tonic contraction present in muscles whose central connections are intact. Their irregularity, and the irregularities which sometimes appear in records from a single end-organ with

a small stimulus (cf. Fig 4) may be accounted for by supposing that the stimulus is very near the threshold value. The excitability of a nerve returns very gradually when recovery is nearly complete and the mapping of this part of the curve is often a matter of great difficulty owing to slight changes in the threshold. So with the end-organ a slight change in the effective value of the stimulus will produce a considerable change in the rhythm of the response the change in rhythm may itself disturb the threshold afresh and in this way an irregular discharge will be produced. In fact the end-organ may have the same difficulty in giving a constant response to a weak stimulus as the experimenter has in plotting the final stages of the recovery curve of a nerve.

Nature of adaptation The gradual decline in rhythm which occurs with a steady stimulus has already been described, but its cause has not been dealt with. It might be due to a gradual decrease in the excitability of the end-organs or to a gradual increase in their refractory period, or to both these causes. If only the former were in action, the effective value of the stimulus would decrease so that longer and longer intervals would have to elapse after each impulse before the end-organ had reached the degree of excitability necessary for the setting up of a fresh impulse. A slowing of the rate of recovery would have the same effect, although the excitability remained unaltered. When a gradually increasing current is applied to a nerve fibre the adaptation which occurs is confined to the mechanism of excitation, for the stimulus may fail to set up an impulse at all if it increases too slowly. Presumably the failure of a constant current to set up more than one impulse is due to the same cause, i.e. to the rapid decline in the stimulating value of the current. The rate of recovery may be altered in the region which becomes adapted, and Brücke⁽⁵⁾ has shown that the refractory period of the nerve fibre or nerve ending becomes prolonged when a rapid succession of impulses are passing but this by itself would not account for the complete failure to set up a second impulse. So if the decline in rhythm from the end-organ is due to a process of adaptation of the same kind as that occurring in the nerve fibre we should expect to find evidence of a change in excitability with or without a change in refractory period in addition.

In order to study the process more thoroughly we have increased the tension on the muscle at varying rates recording both the tension on the muscle at each moment and the frequency of the afferent impulses. The tension was applied by a short lever fixed in the centre of a twisted elastic band. A similar lever at one end of the band was rotated at varying speeds through a known angle and its movement was communi-

cated to a pointer moving across the slit of the film camera. The lever in the middle of the band is fixed to the thread from the muscle and can only move through a very small arc, but when the end lever is rotated the tension on the thread is increased and its final value depends on the amount of movement of the end lever. The tension developed for different movements of the end lever was determined by hanging weights on the centre lever and finding the amount of movement required to raise them. Fig 10 A shows the effects of varying the rate at which the tension increases and the records of another experiment are shown in

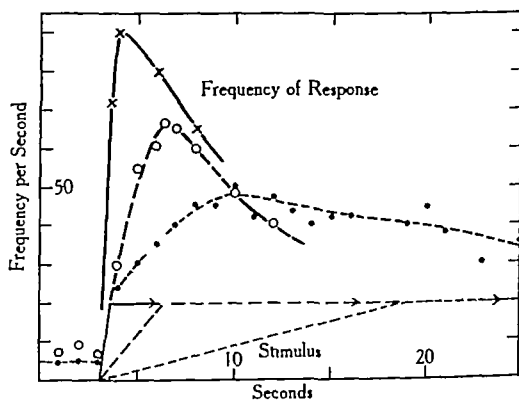


Fig 10 A

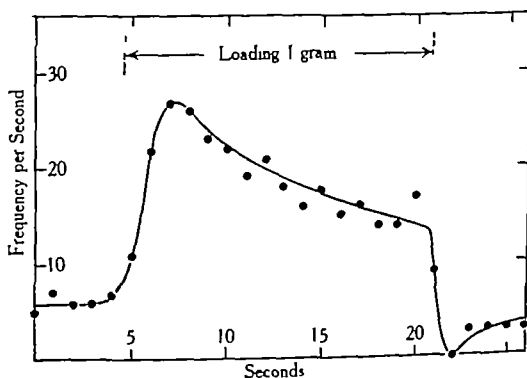


Fig 10 B

Fig 10 A Exp 13 13° C. Frequency of responses with various rates of loading showing effects of adaptation. Maximum tension 1.3 gm

B Exp 12 14° C Frequency before, during and after loading Monophasic waves only

Fig 11 With very rapid loading the maximum frequency is reached at about the same moment as the maximum tension, but with more

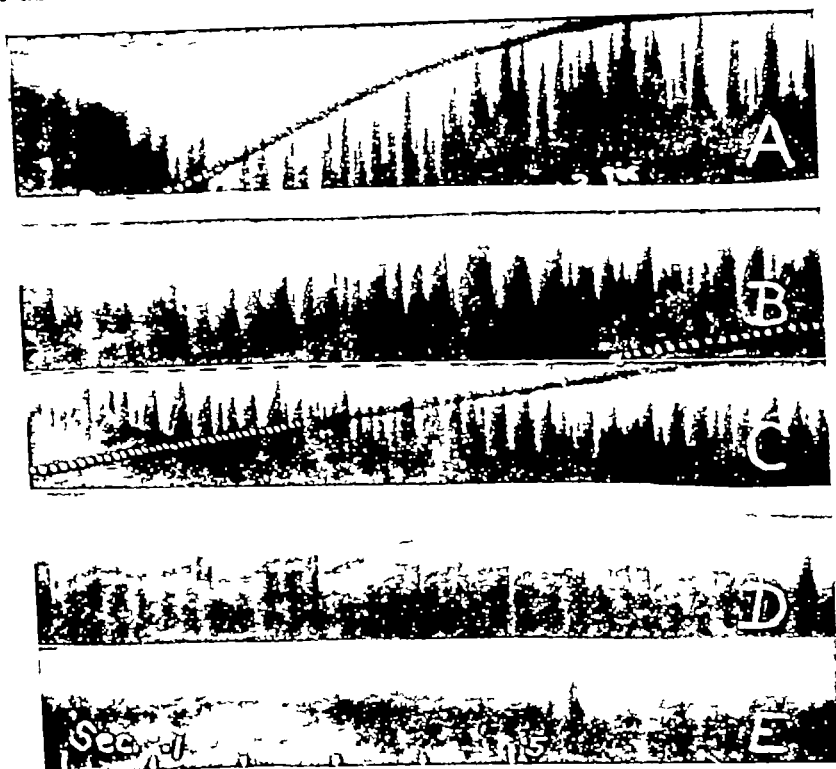


Fig 11 4 B and C Exp 13 Tension of 1.3 grm. applied rapidly in A and slowly in B and C B and C are portions of the same record with an interval of .75 sec omitted.

D and E Exp 12. D immediately before loading impulses about 7 per sec. E immediately after loading Pause without impulses The single excursion is probably an artefact

gradual loading the maximum frequency is much less and it is reached some time before the stimulus is at its full value These curves would be the natural result of any process of adaptation and they merely serve to emphasise the fact that the adaptation begins at the moment that the stimulus is applied A more significant fact is shown in Fig 10 B, which records also the effects of a rapid unloading after the tension had been at its full value for 17 seconds In all our experiments the result of this has been a complete cessation of all impulses for a period varying from .75 to 1.5 sec followed by a gradual return to the normal "resting"

frequency of 3-8 per second. In the present experiment the record immediately before and immediately after the stimulus is shown in Fig 11 *D* and *E*. It is difficult to explain this pause in the discharges without supposing that a true fall of excitability has occurred. During the period of stimulation the frequency has fallen to about half its initial value. If this fall is due to an increase in the refractory period the latter must have risen to about double its initial value, and we should expect to find a discharge at half the initial resting frequency when the stimulus is removed. Actually the initial frequency is 6 per second but there is a pause of 1.3 sec. at the end of stimulation.

We conclude that the decline in the frequency of response is due in part at least to a fall of excitability in the end-organs when the stimulus is continued and their adaptation is thus brought into line with the much more rapid process which occurs in the nerve fibre.

Remarks

The behaviour of the end-organs which we have examined seems to be explained very simply in terms of the general properties of excitable tissues, in particular by the phenomena of adaptation and of the refractory state. It is probable, therefore, that other types of end-organ will be found to react in much the same way to the particular stimuli which call them into play. This probability is strengthened by the close resemblance of the records of impulses in various types of sensory nerve dealt with in Part I, though an investigation of other single end-organs will be needed to establish it. A point of considerable interest is that the frequency of the discharge from the end-organ is so much slower than the maximum frequency which the nerve fibre can tolerate. If this is true of end-organs in general it means that the frequency of the impulses in the fibres of the auditory and of the optic nerves is lower than is often supposed. This again must wait for experimental confirmation. Finally, it is worth enquiring whether the synapses of the central nervous system may not sometimes react in the same way as the end-organ to the mass of impulses reaching them. The regular succession of impulses obtained by Brevée and Dusser de Barenne⁽⁶⁾ from the spinal cord treated with novocaine is a remarkable instance of rhythmic discharge from motor neurones which may perhaps be brought into line with the phenomena dealt with in this paper.

SUMMARY

(1) The afferent impulses produced by the receptors in the sternocutaneous muscle of the frog have been recorded with a capillary

electrometer and three-stage amplifier. The receptors were stimulated by stretching the muscle with different weights. There are generally three or more end-organs in the muscle but they may be reduced to one or two by cutting successive strips from the muscle.

(2) The impulses set up by a single end-organ occur with a regular rhythm at a frequency which increases with the load on the muscle and decreases with the length of time for which the load has been applied. The frequency may vary from 5 to 100 a second, though the very low frequencies are usually irregular.

(3) The regular rhythm can be explained as the natural consequence of the refractory period and the subsequent return of excitability in the end-organ, the difference in the response of a nerve fibre and an end-organ to a constant stimulus depending on the much more rapid adaptation to the stimulus in the former.

(4) The "recovery curve" of the end-organ can be determined by comparing the frequencies with different stimuli. It is found to have the same general form as that of other excitable tissues, but its time relations are about five times as long as those of the nerve. The absolute refractory period is probably about 0.1 sec.

(5) Owing to the more rapid recovery of the nerve fibre the impulses which enter it from the end-organ will always find it almost completely recovered from the effects of the previous impulse.

(6) There is an all-or-nothing relation between the size of the electric response in the nerve fibre and the strength of the stimulus to the end-organ.

(7) The adaptation which occurs when the stimulation is continued is due in part at least to a fall of excitability and it is unlikely that there is much change in the refractory period.

(8) The response of the end-organs in muscle can therefore be brought into line with that of excitable tissues in general. No new properties have to be invoked to explain it, and it seems likely that other types of receptor will be found to react in the same way.

The expenses of this research were defrayed in part by a grant to one of us from the Government Grants Committee of the Royal Society.

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THE ACTION OF IONS UPON INTRA-AURICULAR CONDUCTION IN THE TORTOISE

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A LARGE number of observations have been made regarding the influence of changes in the ionic content of Ringer's fluid upon the conduction of the wave of excitation in the hearts of cold-blooded vertebrates Mines⁽¹⁾, Daly and Clark⁽²⁾, Andrus and Carter⁽³⁾, all agree that the A-V conduction is impaired by increase or decrease of the potassium content, by decrease of the calcium content or by increase of the hydrogen ion concentration. These workers also agree that the A-V conduction is slightly improved by decrease in the hydrogen-ion concentration or by increase of the calcium content.

Andrus and Carter⁽³⁾ studied also the action of ionic changes on the perfused turtle's ventricle. The rate of spread of excitation over the ventricle was estimated by measuring the total length of the Q, R and S waves. Intraventricular conduction was impaired by decrease of C_{Na} or C_K and by increase of C_{Ca} or C_H , it was unaffected by increase of C_K or decrease of C_{Ca} and was improved by decrease of C_{Na} , or decrease of C_H -ion concentration.

The results as regards intraventricular conduction are in some cases difficult to understand and it seems doubtful whether the duration of the QRS waves necessarily gives a measure of the rate of conduction in the ventricle when the electric response is profoundly altered by changes in ionic concentration.

Method The writer has studied the influence of changes in concentration of the kations present in normal Ringer's fluid upon isolated strips of the tortoise's auricle.

The experiments were conducted in the summer and early autumn and 25 large specimens of *Testudo graeca* were used.

Short strips (20 mm) were taken from the right auricle from the sinus venosus to the auricular appendix. Longer strips were obtained by cutting a continuous ring from both auricles, in this way lengths up to 65 mm were obtained. I was unable to observe any significant difference in the

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rate of conduction in the strips cut in these different methods. The strips were pinned out on pieces of paraffined cork carried on glass rods, and were immersed in a bath containing Ringer through which air was bubbled. Records were taken of the mechanical and electrical responses. The mechanical responses were measured by attaching fine hairs at intervals along the strips to light straw levers suspended by threads in the manner described by Mines(a), the movements of which were recorded optically. Records of the electrical variation were taken at two or three points simultaneously, two string galvanometers were used, one of the Cambridge and the other of the Edelmann pattern. Electrodes of the D'Arsonval-Lapicque pattern were used. They were made of silver wire provided with pin-head endings and were freshly coated with silver chloride every day.

Some difficulty was experienced in fixing the electrodes to the auricle. The best method was found to be to enclose the wire in a fine glass tube with the pin-head projecting and to fix it against the auricular strip by a fine spring pressing on the opposite side of the auricle. The leads were connected to the terminals by spirals of fine silver wire which permitted the electrodes to move with the auricle. The pressure was firm enough to prevent the auricle from slipping from under the electrodes during contraction, but not to injure the auricle. At first the pair of electrodes were arranged transversely to the strips as shown in Fig 1 a, but later

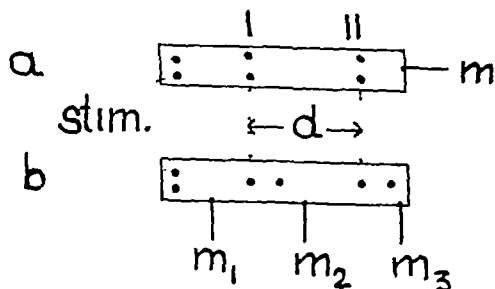


Fig 1 Arrangement of electrodes and levers on the auricular strips I II, first and second pair of electrodes, *stim* stimulation electrodes *d*, distance between electrodes, *m*, m_1 , m_2 , m_3 , points of attachment for recording mechanical response

they were arranged along the length of the strip as shown in Fig 1 b. In the latter case the distances between the electrodes were calculated from the electrode of each pair that was nearest to the stimulating electrodes.

In some cases the auricle was allowed to contract at its natural

frequency, but in most cases artificial rhythm was produced by electrical stimulation, and the moment of stimulation also was recorded. Unfortunately it was not found possible to keep the frequency constant during all experiments because a certain minimal rate of artificial stimulus was essential in the normal auricle to prevent the occurrence of spontaneous contractions, and this frequency was often greater than the auricle could follow after poisoning with ionic changes.

The distance between the electrodes could be measured with an error of ± 5 p c, and the time between the electrical response could be measured with an error of ± 2 p c. The measurements of conduction, therefore, have a possible error of ± 7 p c.

The normal Ringer used had the following percentage composition: NaCl 0.65, KCl 0.015, CaCl_2 anhyd. 0.012, NaHCO_3 0.05. Fresh glass distilled water was used. The hydrogen-ion concentration was pH 8.0. A stream of air was bubbled through the solution, and in order to prevent considerable changes in hydrogen-ion concentration, the fluid in the vessel was changed in short intervals.

During a series of experiments the electrodes remained in their position unchanged. Before the electrodes were connected to the galvanometers, the vessel was emptied and both strip and electrodes dried quickly with filter paper. The type of record is shown in Figs. 6 and 7.

The normal rate of conduction and its variations

H. Fredericq(4) showed that the form of the auricular electrogram of the tortoise does not differ essentially from the ventricular electrical response. Records taken at low speeds confirm his statement, the electrogram consists of a rapid diphasic initial variation, which is followed by a much slower final one.

To the amplitudes of the deflections and the duration of the electrical response under varied conditions no particular importance was attached, for the object of the experiments was to ascertain as accurately as possible the commencement of the electrical variation at each electrode.

Different animals showed great differences in the rate of conduction in normal Ringer. Such rates varied from 30 to 290 mm per second, but fortunately most fell between the limits of 90 and 120 mm per second. The reason for this variation is not clear but the specimens with the highest conduction rates also showed the largest mechanical responses, and therefore the rate of conduction is probably dependent on the condition of the tortoise.

Lewis(5) and Fredericq(7) both observed variations from about

100 to 300 mm/sec in the conduction rate of the tortoise's ventricle, and after prolonged isolation considerable reductions in both the force of contraction and rate of conduction were observed, as is shown in Table I

TABLE I Temperature 16° C No stimulation.

Time since excision of the heart h. m.	Frequency per min	Distance of electrodes mm.	Interval between responses at a and b in secs	Rate of conduction		Height of contraction	
				mm./sec	p c	mm.	p c
6 26	10	23	0 08	288	100	47	100
28 10	10	19	0 144	131	45	25	53

Table II shows that variations in the normal conduction of a single strip observed during a shorter interval of time do not much exceed the limits of experimental error

TABLE II. Ventral strip of the auricle Normal Ringer Conduction from the right to the left auricle No stimulation Height of mechanical contraction 40 mm Frequency 20 per minute

Time since excision of the heart h. m.	Time of action of the fluid	Distance of electrodes a and b in mm.	Interval between responses at a and b in secs.	Rate of conduction mm/sec
2 7	5	25	0 28	89
2 26	5	29	0 326	89
2 38	6	29	0 36	81
5 11	20	29	0 32	83
5 30	8	29	0 336	86

Freshly excised auricular strips at first conduct badly, but conduction improves after the strip has been kept in aerated Ringer for a time ranging from 30 minutes to 2 hours The following example illustrates this

TABLE III. Distance of electrodes 16 mm Temperature 18° C Frequency 15 per minute

Time since excision of the heart h. m.	Rate of conduction mm./sec.
1 10	91
2 42	105
4 55	116

Influence of temperature on rate of conduction

For investigations on effect of changes of temperature the vessel containing the preparation was put into a box, the walls of which were lined with cotton wool, a narrow slot on one side allowed access to the strip The temperature of the air inside of the box could be kept constant for some minutes, whilst electrograms were taken, by a large water bath

surrounding the vessel and by a system of lead tubing through which water of the required temperature passed. The air bubbling through the Ringer's fluid was brought to the desired temperature.

TABLE IV Auricular strip Normal Ringer Three pairs of recording electrodes, stimulating electrodes to 1=7 mm, distance 1-2=16 mm, 2-3=23 mm.

Time since excision of the heart h. m	Temp ° C	Fre quency per min	Time of action min.	Interval (sec)			Rate of conduction			Height of me chanical contrac tion (mm.)
				st-1	1-2	2-3	st-1	1-2	2-3	
4 55	17.7	23	30	0.04	0.14	0.35	178	116	106	25
5 15	10	23	7	0.06	0.21	0.48	117	77	86	23
5 25	27	40	10	0.03	0.13	0.3	224	128	130	20

The succession of events in the strip is represented graphically in Fig. 2. The dotted lines show the transmission of the wave of mechanical

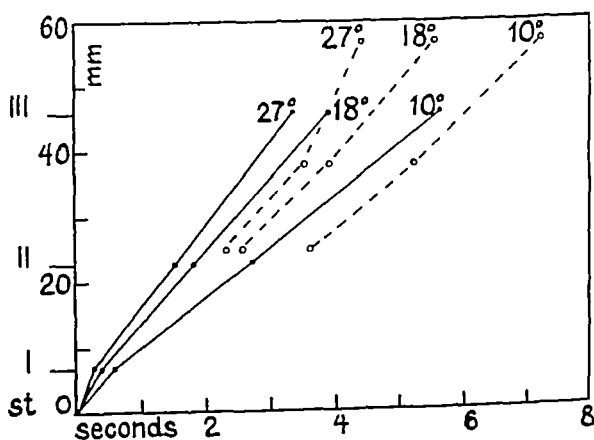


Fig. 2 The rate of conduction of the wave of excitation in the tortoise auricle at various temperatures. Ordinates distance between electrodes. Abscissae time in seconds. Continuous lines represent passage of wave of electrical variation. Dotted lines represent passage of wave of contraction.

contraction, which follows the spread of electrical changes on a nearly parallel course. The distance of both lines on any point of the ordinate gives the mechanical latent period, which is about 0.6–0.3 second. The value for the electrical latent period, if there is any, falls in the limits of experimental error.

The value for $Q/10$ calculated from the intervals between the stimulation electrode and the third pair of recording electrodes is, between 10° and 17.7°, 1.5, and between 17.7° and 27°, 1.2. These figures agree with those found by other observers in the hearts of amphibia and

reptiles Clark(1) and Daly and Clark(2) measured the P-R interval in frog's heart, the value of the temperature coefficient of conduction is $Q/10 = 1.6$ between 18° and 24° and 15° and 26° respectively Ishihama(3) found for the transmission of the mechanical contraction in the frog's heart kept at constant rate an average value $Q/10 = 1.8$ over a range of temperature from 5° to 20° , this temperature coefficient, however, is lower at higher temperatures and can be as low as 1 Spadolini(4) measured the P-R interval in the hearts of *Triton cristatus* and *Emys* at 2° and 30° C and his results show a $Q/10$ of 1.45 and 1.25 respectively In this case the frequency of the hearts was allowed to vary Amsler and Pick's data(5) show a far higher $Q/10$ for the A-V interval ($Q/10$ 25° - 30° C = 4.8) These last figures depend, however, on mechanical records which are less exact than electrical records

Alterations in hydrogen-ion concentration

The pH of Ringer's fluid was altered by adding a small amount of 1/M NaOH or 1/M HCl and then determined colorimetrically The limits of alkalinity and acidity tested were pH 11 and pH 4.5 The results are given in the table below and in Fig 3, which shows that both acid

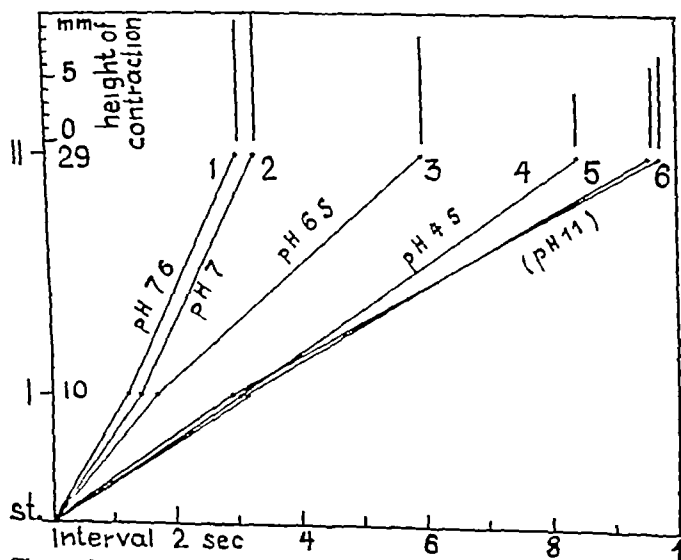


Fig 3 The conduction of the wave of excitation, and the height of mechanical contractions at varying concentrations of hydrogen ions Ordinates and abscissae as in Fig 2

and alkali depress conduction and that the rate of conduction is depressed uniformly throughout the strip

TABLE V Auricular strip Distance of electrodes stimulation—1 pair=10 mm.,
1-2 pair=19 mm Frequency 21 per minute Temperature 18°-20° C.

Time since excision of the heart h m	Fluid	Time of action min.	Interval sec		Rate of conduction mm/sec		Height of mechanical contrac tion (mm)	Fig 3
			st.-1	1-2	st.-1	1-2		
27 6	Normal Ringer, pH=8	6	—	0 144	—	131	25	—
27 45	Alkaline Ringer, pH=11	2	Nil	Nil	Nil	Nil	—	—
	Normal Ringer	13	0 29	0 08	34	28	7	(0)
28 10	Ringer, pH= 7 6	11	0 13	0 18	79	109	10	(1)
29 19	Ringer, pH= 7 0	33	0 14	0 19	70	100	10	(2)
29 35	Ringer, pH= 6 5	10	0 17	0 43	58	44	8	(3)
29 44	Ringer, pH=45 0	2	0 30	0 54	33	35	4	(4)
30 20	Ringer, 0 048 p c CaCl_2	20	0 33	0 617	30	30	6	(0)

Alterations in calcium and potassium content

Lack of calcium and excess of potassium decrease the height of contraction, but these ionic changes act on the conduction in a different

TABLE VI. Auricular strip Distance of electrodes 12 mm No stimulation.
Temperature 20° C

Time since exposure of the heart h. m	Fluid	Time of action min	Frequency per min.	Interval sec.	Rate of conduc tion mm./sec	Height of contrac tion mm.	Fig 4
2 5	Normal Ringer	30	15	0 09	133	75	(1)
2 55	Excess KCl (0 06 p c KCl)	7	10	0 23	52	45	(2)
3 10	Normal Ringer	9	15	0 07	171	70	(3)
3 44	Excess KCl (Normal Ringer)	25 (28)	10	0 48	25	40	(4)
4 51	Ringer minus CaCl_2	21	10	0 08	150	21	(7)
5 43	Excess CaCl_2 (0 048 p c CaCl_2)	30	12	0 055	220	65	(8)
6 10	Normal Ringer	25	13	0 055	220	—	—
7 52	Normal Ringer	30	14	0 063	190	70	—

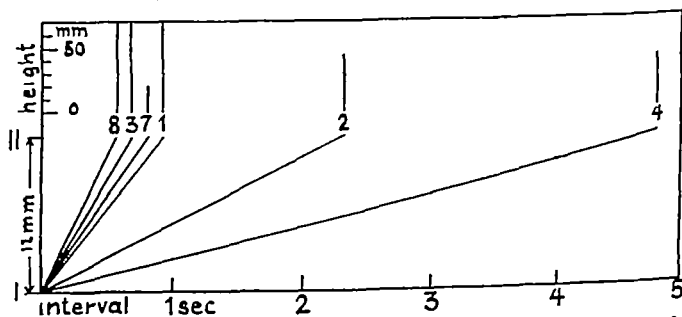


Fig 4. The conduction of the wave of excitation and the height of the mechanical response with varying concentrations of calcium and potassium 1 and 3, normal Ringer 2 and 4, Ringer containing excess KCl (0 06 p c.), acting for 7 and 25 minutes respectively 7, calcium free Ringer acting for 21 minutes. 8, Ringer containing excess of CaCl_2 (0 048 p c)

manner The rate of conduction is immediately decreased by increase of the KCl concentration and this effect increases progressively the longer the excess of KCl acts, whilst the conduction after removal of calcium is not impaired at all, even after prolonged action. These effects are shown in Table VI and Fig 4 above

Fig 4, in which this experiment is represented graphically, shows that the value for the rate of conduction after removal of calcium falls between those determined for the normal conduction. It shows also the great discrepancy of the alteration of height of mechanical response and electrical conduction after poisoning with potassium and washing out of calcium respectively This relationship can be clearly seen in Fig 5,

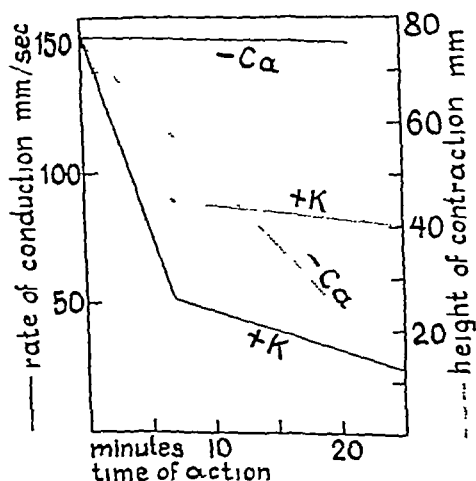


Fig 5 The effects produced on the rate of conduction and force of contraction by excess of potassium and lack of calcium. Continuous lines rate of conduction of wave of electrical variation in mm per sec (normal rate = 152 mm./sec) Dotted lines height of mechanical response

where the rate of conduction and the height of mechanical contraction (dotted lines) are plotted against the time of action Fig 6 shows the typical curves obtained from the action of excess of potassium.

Table VII shows the relative action of excess of potassium on rate of conduction and height of mechanical contraction. The average result of 0.06 p.c. KCl in nine experiments was to reduce the rate of conduction 69 p.c. and the height of mechanical contraction 59 p.c.

TABLE VII. Action of excess of potassium on conduction and contraction.

No of preparation	Concentration of KCl p c	Time of action mm	Rate of conduction	Height of mechanical contraction
(Normal)	0 015	—	100	100
XX	0 03	2	79	61
XXI	0 06	0 5	78	50
XX	"	2	50	20
X	"	7	39	60
XX	"	2	35	26
XVII	"	12	21	64
X	"	25	19	55
XIX	"	13	19	12
XVII	"	21	14	51
VIII	0 06	20	7	33
XVII	0 15	2	9	11

(The figures for rate of conduction and height of mechanical contraction are expressed as percentages of the average figures observed in normal Ringer)

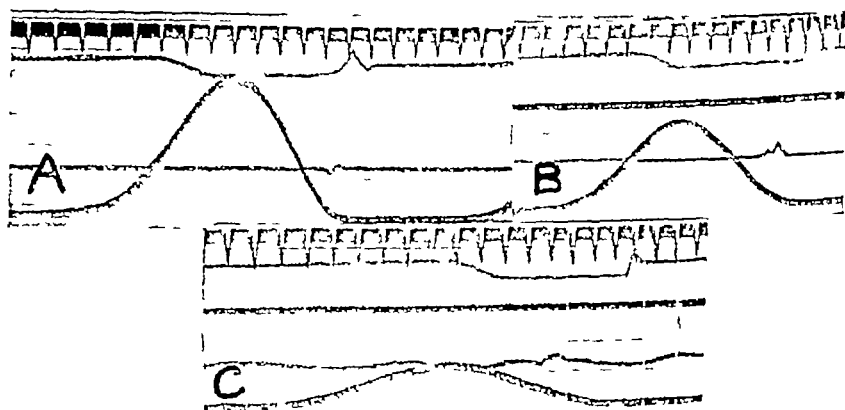


Fig 6 Action of excess of KCl on conduction and contraction A, normal, B, excess of KCl (0 03 p c) for 2 minutes C, excess of KCl (0 06 p c) for 4 minutes The tracings read from left to right and the curves from above downwards record (1) time, $\frac{1}{4}$ second (2) and (4) electrical variation at electrodes 10 and 34 mm distance respectively from stimulating electrodes (3) stimulus (absent in A), (5) mechanical response

The difference between the effects of excess of KCl and lack of CaCl_2 is clearly shown in Table VIII. It will be seen that when a 50 p c reduction in the height of mechanical response is produced by excess of potassium the rate of conduction is reduced to 14 p c of the original rate, whereas when 80 p c reduction in the height of mechanical response is produced by lack of calcium the rate of conduction is unaltered

TABLE VIII (a-b) Action of excess potassium and lack of calcium on the rate of conduction.

Time since exposure of the heart h. m	Fluid	Time of action min.	Frequency of contraction per min.	Distance of electrodes a-b mm	Interval between response of a-b sec	Rate of conduction mm/sec	Height of contraction (mm)
<i>Expt 1:</i>							
6 26	Normal Ringer	18	9.2	23	0.08	288	47
6 54	Excess KCl (0.06 p.c.)	21	10.7	„	0.59	39	24
7 43	(Normal Ringer)	(30)	—	—	—	—	—
	Excess KCl (0.15 p.c.)	2	3.8	„	0.86	27	5
8 20	(Normal Ringer)	(10)	—	—	—	—	—
	Ringer minus CaCl ₂	8	11.5	19.5	0.067	291	20
<i>Expt 11:</i>							
24 20	Excess CaCl ₂ (0.048 p.c.)	3	18	24	39	61	67
24 33	Ringer minus CaCl ₂	5	17	„	38	63	12
28 00	Normal Ringer	9	19	„	405	61	
28 11	Ringer minus CaCl ₂	5	19	„	34	70	13.5
					336	72	3
					36	66	3

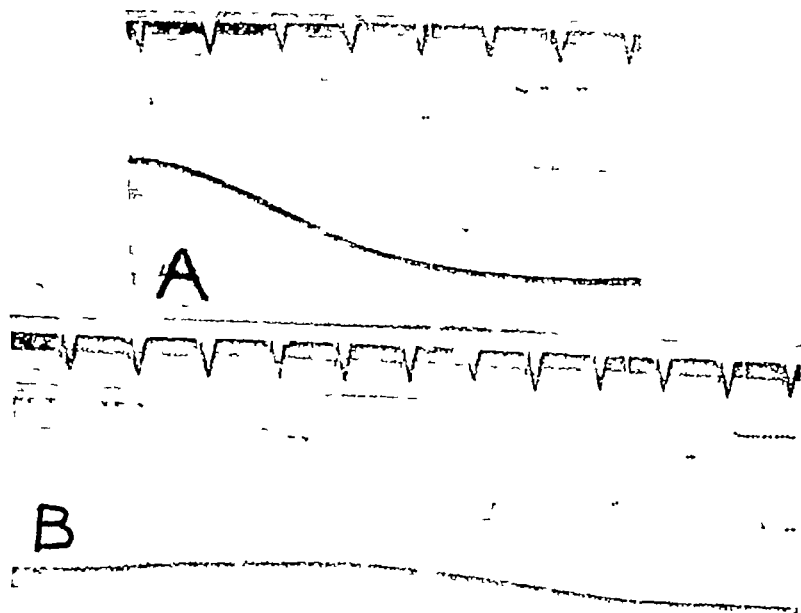


Fig 7 The action of lack of calcium on conduction and contraction. A, normal Ringer, B, calcium free Ringer for 5 minutes. The curves read from right to left and show from above downwards (1) time, $\frac{1}{2}$ second (2) and (3) the electrical variations at electrodes 24 mm apart, (4) mechanical response

Increase in concentration of calcium to 0.048 p.c. CaCl_2 or omission of CaCl_2 , caused no appreciable effect on the rate of conduction in strips in good condition. In strips, in which the spread of the electrical variation had been impaired by excess of potassium, acid, alkali or alcohol, the conduction could be restored to a normal figure by an excess of calcium.

The relative effects of ionic changes on the rate of conduction and on the height of contraction of the tortoise auricle can be summarised as follows

TABLE IX. Effect of ionic changes on the auricle of the tortoise
- = depression, 0 = little effect

Alteration in ionic concentration	Mechanical response	Conduction of electrical variation
- Ca	-	0
+ K	-	-
+ H^+	-	-
- H^+ (+ OH -)	-	-

Discussion

Numerous workers have concluded that lack of calcium has a special depressant effect on the mechanical response of the heart and produces much less effect on the chemical and electrical changes. Locke and Rosenheim(10) showed that a mammalian heart perfused with a fluid containing no calcium continued to consume sugar at almost the normal rate.

Locke concluded that "calcium is necessary for the conversion of the heart's chemical energy into the mechanical energy of its beat." Mines(1) showed that in the cold-blooded heart arrested by lack of calcium regular spontaneous electrical variations of normal form and extent could be observed. Daly and Clark(2) stated that the conduction of the electrical variation in frog's heart remained unaffected by a reduction of the calcium content. Daly and Clark pointed out that lack of calcium and excess of potassium produced almost identical effects upon the mechanical response of the frog's heart, but quite different effects on the transmission of the wave of excitation. They found that excess of potassium depressed the conduction very powerfully, while removal of calcium had only a slight action in this respect.

The results obtained with direct measurements of the rate of conduction in the auricle of the tortoise show that excess of potassium produces a stronger depressant action on the rate of conduction than on the height of the mechanical response, whereas lack of calcium can almost

completely abolish the mechanical response without affecting the rate of conduction

Mines⁽¹⁾ and Daly and Clark⁽²⁾ thought that normal electrical responses were obtained from the heart when the mechanical response had been abolished completely by lack of calcium. Einthoven and Hugenholtz⁽¹¹⁾ and Arbeiter⁽¹²⁾ showed that if a sufficiently delicate method of recording mechanical movements were employed, mechanical movements could be demonstrated in hearts which had been perfused for 24 hours with calcium-free Ringer. They also concluded that in every case the mechanical and electrical responses varied in a strictly parallel manner.

My results show that different ionic changes produce very different effects on the electrical and mechanical response. Excess of potassium, which acts chiefly on rate of conduction of the wave of excitation, affects the form of the electrical response profoundly, whereas lack of calcium which does not affect the rate of conduction produces little effect on the form of the electrical response.

I did not measure the potential of the electrical changes which occurred in strips after the various ionic changes, and therefore express no opinion as to whether the height of mechanical response and the changes in electrical potential vary in a parallel manner.

My experiments show however that mechanical contraction can be reduced to less than 10 p c of its original amplitude without any change occurring in the rate of conduction of the wave of excitation, and this proves that these two factors can vary independently.

My experiments support the view that calcium and potassium are not true antagonists, but act upon different functions of the heart.

SUMMARY

(1) The rate of conduction of the wave of excitation in the auricle of the tortoise, calculated from measurements with a direct method, increases with increase of temperature. The temperature coefficient $Q/10 = 1.5$ between 10° and 17.7° C and $Q/10 = 1.2$ between 17.7° and 27° C.

(2) The conduction is decreased by both increase and decrease of the optimal hydrogen-ion concentration $pH = 7.0-8.0$.

(3) Opposite changes in ionic concentration, decrease of calcium and increase of potassium content, which produce a similar effect upon the mechanical response, do not act in the same sense upon the conduction.

Increase in concentration of calcium to 0.048 p.c. CaCl_2 or omission of CaCl_2 , caused no appreciable effect on the rate of conduction in strips in good condition. In strips, in which the spread of the electrical variation had been impaired by excess of potassium, acid, alkali or alcohol, the conduction could be restored to a normal figure by an excess of calcium.

The relative effects of ionic changes on the rate of conduction and on the height of contraction of the tortoise auricle can be summarised as follows:

TABLE IX. Effect of ionic changes on the auricle of the tortoise
- = depression, 0 = little effect

Alteration in ionic concentration	Mechanical response	Conduction of electrical variation
- Ca	-	0
+ K	-	-
+ H^+	-	-
- H^+ (+ OH -)	-	-

Discussion

Numerous workers have concluded that lack of calcium has a special depressant effect on the mechanical response of the heart and produces much less effect on the chemical and electrical changes. Locke and Rosenheim(10) showed that a mammalian heart perfused with a fluid containing no calcium continued to consume sugar at almost the normal rate.

Locke concluded that "calcium is necessary for the conversion of the heart's chemical energy into the mechanical energy of its beat." Mines(1) showed that in the cold-blooded heart arrested by lack of calcium regular spontaneous electrical variations of normal form and extent could be observed. Daly and Clark(2) stated that the conduction of the electrical variation in frog's heart remained unaffected by a reduction of the calcium content. Daly and Clark pointed out that lack of calcium and excess of potassium produced almost identical effects upon the mechanical response of the frog's heart, but quite different effects on the transmission of the wave of excitation. They found that excess of potassium depressed the conduction very powerfully, while removal of calcium had only a slight action in this respect.

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(3) Opposite changes in ionic concentration, decrease of calcium and increase of potassium content, which produce a similar effect upon the mechanical response, do not act in the same sense upon the conduction.

The conduction of electrical variation is greatly impaired by an excess of potassium and scarcely altered by lack of calcium

(4) These results support the view, that calcium is intimately concerned with mechanical response as opposed to conduction of the wave of excitation

I should like to take this opportunity of thanking Prof A J Clark for granting me the facilities of the laboratory and his advice and help in the experiments

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THE VASO-DILATOR ACTION OF HISTAMINE, AND ITS PHYSIOLOGICAL SIGNIFICANCE

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THE histamine paradox, which has been the subject of much discussion, may be stated thus briefly. In the cat, which has been the usual subject for experiment on its action, histamine regularly produces, under anæsthesia, a powerful depressor effect, having all the characteristics of a general vaso-dilatation, in perfused organs from the same species histamine has been found habitually to produce, not vaso-dilatation, but vaso-constriction in its place. Explanations of widely different kinds have been offered for this paradox. Dale and Richards(1) satisfied themselves that the effect seen in the anæsthetised cat was a true, peripheral vaso-dilatation, independent of the nervous system. They explained the paradox by the incidence of the effect on the capillaries rather than the arteries, and showed that, under appropriate conditions (presence of blood corpuscles and adrenaline), the vaso-dilator effect could be demonstrated even on the artificially perfused organs of the cat. Other observers have attempted to explain it by supposing that the depressor effect seen *in vivo* was not due to vaso-dilatation. Thus Mautner and Pick(2) attributed it to constriction of hepatic venules McDowall(3), while admitting capillary dilatation as a factor in the effect, has suggested that constriction of pulmonary arterioles is the earliest and principal agent in the depressor action. Inchley(4) has attributed the effect to constriction of the venules.

In the present paper we present the results of observations undertaken primarily to complete those of Dale and Richards. It was important to know whether the action of adrenaline, mentioned above, was a specific one, or whether other vaso-constrictor agents could replace it, in creating a condition favourable to the vaso-dilator effect of histamine in artificial perfusion. The observations have been extended to other species, and some further experiments have been made on the effects seen with natural circulation.

PART I EFFECTS OBSERVED WITH ARTIFICIAL PERFUSION

METHODS

We constructed a perfusion scheme, having several points of superiority to the somewhat makeshift apparatus which Dale and Richards had at their disposal. It consisted essentially of the following parts, the arrangement of which can be seen from the diagram

(1) The arterial reservoir, *R*, to which the blood was returned after oxygenation, and in which an oxygen pressure was maintained, which could be raised or lowered to any desired level by the adjustable mercury trap, *T*

(2) A glass spiral, *S*, leading from *R* through a vessel of water kept at from 41° to 45° C, and serving to warm the blood on its way to the arterial cannula, *AC*

(3) An electro-magnetic hammer, *H*, by which the thin-walled rubber tube leading from *S* to *AC* was rhythmically compressed, *H* being actuated by a rotatory key, so that the pressure was rendered pulsatile, with a rhythm of about 100 per minute

(4) An outflow recorder of Condon's type(5), *OR*, into which the blood from the vein cannula, *VC*, was led, and which discharged into a large funnel, *F*, from which the blood passed to the oxygenating chamber, *OC*

(5) The oxygenating chamber, *OC*, which in the form finally adopted, and used in all the later experiments, was built on the principle of that described by Hooker(6), and later used by Drinker(7) and others. It consisted of a vertically placed glass cylinder, about 45 cm long and 8 cm wide. This was narrowed at the lower end to a neck closed by a rubber cork, through which passed the tubes for admitting oxygen and withdrawing the blood. The wide upper orifice was closed by a vulcanite lid, *L*. A short tube through this delivered the venous blood near to the centre of a horizontal vulcanite disc, *D*, kept rotating by a small electric motor. The blood, being thrown centrifugally on to the inner surface of the glass cylinder, flowed down this in a thin film, without frothing, to collect in a pool at the lower end, from which it was withdrawn by an adjustable pump, *P*, which lifted it again to *R*. The oxygen tube opened well above the highest level reached by the collecting blood, and the gas passed freely up through the cylinder, escaping by the openings in the lid. The pump and valves were of glass. A spiral of silver wire lightly smeared with vaseline, attached to the opening of the tube which returned blood to *R*, prevented frothing.

It will be obvious that, with such a system, perfusion is carried out

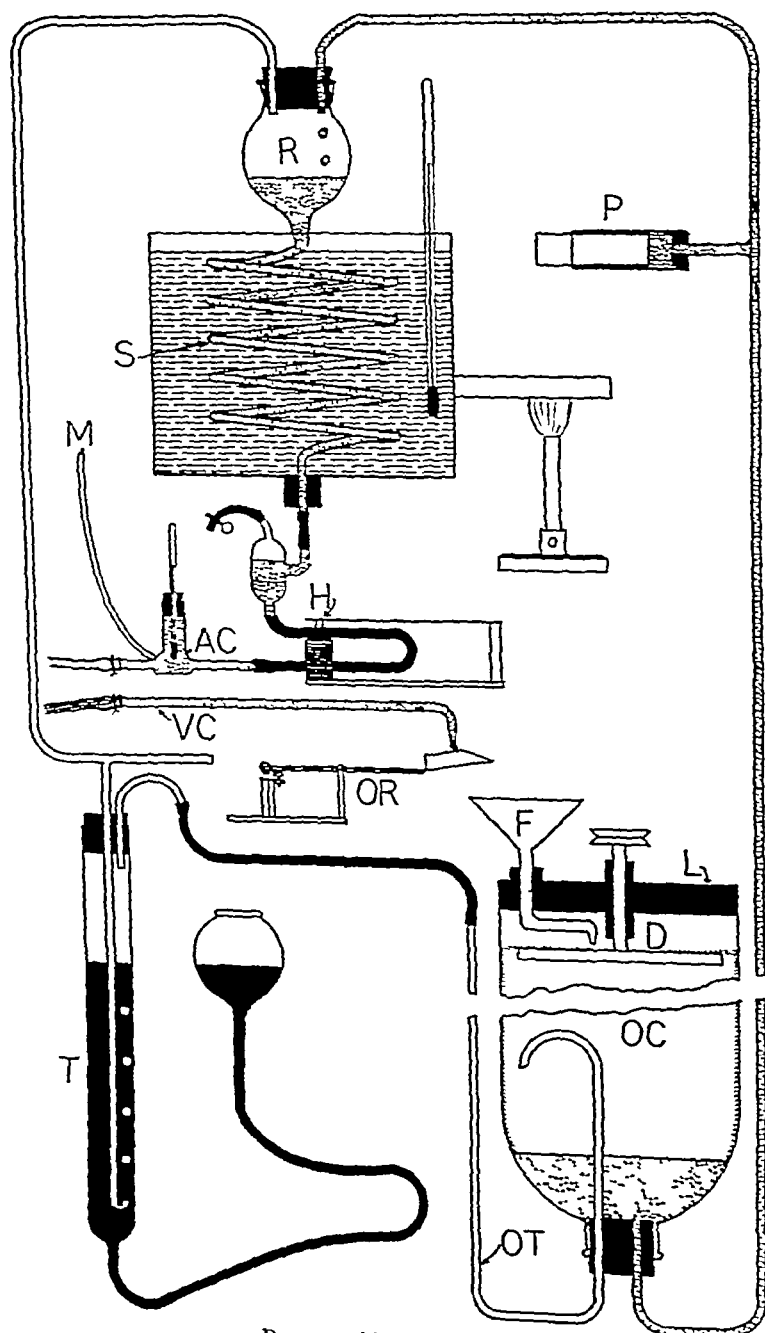


Diagram of Perfusion Scheme.

with a head of pressure, rhythmically cut off by the hammer. The records from the manometer, attached to the arterial cannula, show that the sustained, but pulsatile pressure so produced is very similar to the natural arterial pressure. It has, from our point of view, the advantage that it is but slightly altered by changes in the peripheral resistance, so that the effects of these on the venous outflow and the volume of the perfused organ are practically uncomplicated by secondary changes in the arterial pressure.

The blood used was that of the species, and usually that of the individual, from which the leg for perfusion was obtained. *Hirudin* being no longer available, a few experiments were made in which coagulation was prevented by heparin or by novirudin (a humic acid preparation), on the supposition that defibrination of the blood might interfere with the demonstration of the effects we desired to study. Experience showed that this supposition was not justified, and we subsequently adopted defibrination as a routine procedure. Under ether anaesthesia the animal was bled through a cannula in the abdominal aorta (cat or monkey) or carotid artery (dog). A fairly complete exsanguination was secured by injecting Ringer's solution into the jugular vein in the later stages, about 50 c c being so injected into a large cat and 100 c c into a dog. The blood was defibrinated by stirring with feathers and filtered through cotton-wool. About 150 c c of blood sufficed to fill the apparatus, and this quantity, slightly diluted by the saline infusion, could easily be obtained from a large cat. When smaller cats were used, two were killed to obtain blood for the perfusion of a limb from one.

When Ringer's solution with gum was used it was prepared by a modification of the method recommended by Bayliss⁽⁸⁾. A 20 p c solution of gum arabic in water was made, and to this was added phosphoric acid in the proportion of 0.0268 c c of *M*/10 acid per c c. The mixture was then brought to the alkaline side of the neutral point ($\text{pH} = 7.4$) by addition of normal sodium hydrate, and, after standing awhile to aggregate, was filtered. To the clear filtrate was added 0.9 p c of NaCl. One part of this strong gum-saline was diluted with two parts of ordinary Locke's solution, so that the mixture contained the ions of Locke's solution in approximately normal proportions, and, in addition, between 6 and 7 p c of gum. It was thoroughly saturated with oxygen before introduction into the apparatus.

Cannulae were tied into the femoral artery and vein without undue delay, but experience showed that great hurry was not needed. The external pudic and deep femoral vessels were tied off, and other branches

to the thigh muscles in many instances, the abdominal viscera were removed, and the body wall mass-ligatured with strong twine in three sections. The body was then transected above the mass-ligatures and the spinal canal firmly plugged with plasticine. As perfusion proceeded there was usually some loss of blood into the tissues above the area deliberately perfused, but this was minimised by the precautions mentioned to close anastomotic paths. The cannulae being connected to the perfusion apparatus, the perfusion was started. The plethysmograph was then pushed on and filled, during observation of the venous outflow, care being taken that the cuff exercised no pressure sufficient to interfere with the freedom of circulation. The plethysmograph used was a glass cylinder with an invaginated cuff of soft rubber, making water-tight contact with the vaselined leg. It was filled with warm water, the temperature of which was maintained by an adjacent carbon-filament lamp. The plethysmograph, when adjusted, was clamped firmly to the table. Air connection was made with a small Brodie's bellows.

Injections were made directly into the arterial cannula, the needle being pushed through the rubber tube leading to it. Usually the drug was dissolved in Ringer's solution, in such concentration that the doses used were contained in from 0.05 to 0.2 c.c. The 1 c.c. syringe, containing this small volume, was allowed to fill with warmed blood under the pressure in the arterial cannula, and the mixture was then returned into the blood-stream. In order to ensure that even this slight dilution did not produce fallacious small accelerations of venous outflow, owing to reduced viscosity of the circulating blood, the effects were repeatedly controlled with solutions made up with a small volume of blood taken from the apparatus, instead of with Ringer's solution. The effects were not altered in any way by this procedure, nor did the similar injection of corresponding volumes of plain Ringer's solution produce any perceptible effect. When perfusions were made with gum-Ringer solution, the drugs were dissolved for injection in a sample of this solution.

The lines of record on the smoked paper showed in each case, (1) the leg-volume, (2) the perfusion pressure, (3) the rate of venous outflow, (4) the time in intervals of 10 seconds.

RESULTS

(1) *Perfusion of the cat's leg*

(a) *Perfusion with plain blood* Dale and Richards had found that neither plain blood, nor gum-Locke solution containing adrenaline, would maintain a tone in the peripheral vessels which histamine would

relax Only when red corpuscles and adrenaline were present together in the perfusing fluid did they observe the vaso-dilator effect of histamine Our original object was to test the possibility of replacing adrenaline in this combination by other vaso-constrictor agents The investigation has been given a somewhat different form by the fact that, when blood was used, we obtained evidence of a vaso-dilator effect of histamine, before any artificial addition of adrenaline or other vaso constrictor substance was made, in all but the first experiment of the present series The results in this first experiment correspond with those of Dale and Richards, histamine producing apparently a simple vaso constriction during perfusion with plain blood, and a simple vaso dilatation after adrenaline had been added and the perfusion pressure suitably raised In every other case the first small injection of histamine (usually 0.005 mgm), and in many cases several succeeding similar injections, have produced a decided acceleration of the venous outflow, though blood unmodified, save by whipping or adding heparin, was being used for the perfusion

The plethysmographic changes accompanying this acceleration have been less constant, especially in the earlier experiments on the leg with skin intact Out of the first 16 experiments of this kind, in only five was the acceleration of outflow accompanied by a significant increase of the leg volume, while in some cases a slight diminution of volume was apparent An explanation of this discrepancy was suggested by the results of five other experiments, in which we perfused the leg from which the skin had been removed, the foot being amputated at the ankle joint All severed vessels were carefully ligatured, and for these experiments the plethysmograph was filled with warm Locke's solution instead of water In this series a first small dose of histamine produced in every case a definite increase of volume, accompanying the acceleration of venous outflow and subsiding as it disappeared Dale and Richards had shown that, in the intact animal also, the dilator effect of histamine is exhibited more regularly in the vessels of the muscle than in those of the skin In recording the volume of the perfused leg it is not practicable to include in the plethysmograph the whole of the perfused portion, and, when the skin is left on, the part in the plethysmograph necessarily includes the foot, with much skin and little muscle, while the part outside consists largely of thigh muscle Under such conditions, if we suppose that the vessels of the skin pass more readily than those of the muscles into the condition in which histamine can no longer produce its vaso-dilator action, but merely constricts the arteries, it is easy to under-

stand the production, on balance, of an accelerated venous outflow from the whole area perfused, with no expansion, or even a small shrinkage, of the part yielding the volume record

Our more recent experience, however, has shown that even in the limb with skin intact, perfused with plain blood, it is possible to demonstrate in practically every case the complete vaso-dilator phenomenon—accelerated venous outflow and expansion of volume—in response to a small injection of histamine given at the right period of the perfusion. In our last five experiments, succeeding the series of 16 above mentioned, we have observed it in every case. Figs 1 *A* and 2 *A* show records

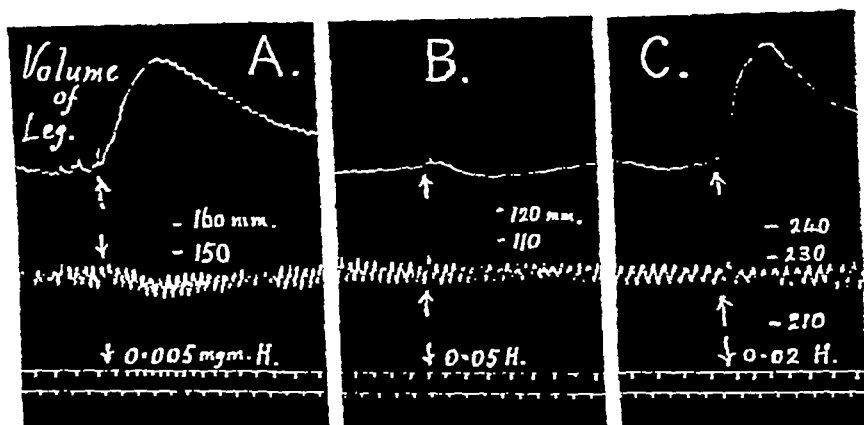


Fig 1 Perfusion of cat's leg with whipped blood. Effects of histamine, (*A*) during initial vascular tone, (*B*) later (*C*) again later, after adding 1 in 5 millions adrenaline. In this and other figures *AC*=acetyl-choline *H*=histamine

obtained from a leg with the skin intact, and from skinned leg muscles respectively. The effects appear to be those of perfectly normal vasodilatation, such as could be expected from the effects of histamine seen in the living animal. Fig 2 *A* shows, for comparison, the effect of a small dose of acetyl-choline, the histamine effect differs from this in its time-relations, but is comparable in all other obvious respects.

The possibility being thus demonstrated of observing the vasodilator action of histamine in an organ perfused with plain blood, it is natural to enquire as to the reason of our present success, and of the uniform failure of earlier workers, including one of us in association with others. We are not confident of being able to give a complete answer. Speed in completing the preparation, and beginning the artificial after cessation of the natural circulation, is certainly not a factor. Dale and

Richards found that transfer from one to the other without even momentary interruption was not effective. We have begun by exsanguinating the animal, and have then made the necessary dissections

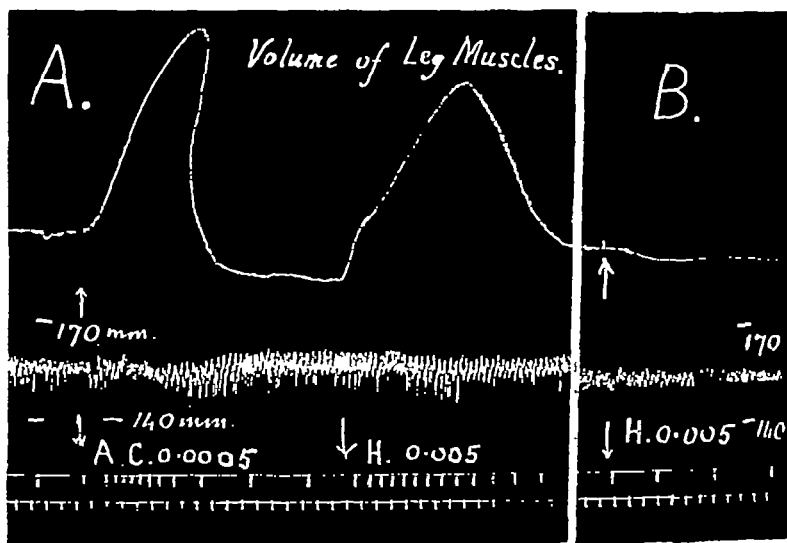


Fig 2 Perfusion of skinned leg with whipped blood. Effects of acetyl choline, and of histamine (A) early in the perfusion, (B) later, after a larger dose of histamine.

and adjustments without hurry, though without undue delay. The tissues have regularly been without circulation for some 15-20 minutes. In our later experiments we have been more careful to tie off such branches in the thigh as could easily be reached, so as to make the part perfused correspond more nearly with that in the plethysmograph, and have probably been more successful in securing a fit of the plethysmograph without pressure. These measures have probably played a part in producing a closer correspondence between the recorded changes in volume and in outflow, but they do not explain the acceleration of outflow regularly observed in practically all the experiments.

So far as we are able to judge, the chief factor in the success of our demonstration has been as follows. Having a perfusion scheme well under control, and working with a minimum of attention, we have been able to begin the record practically with the start of the perfusion and to watch the progress of events. At the outset the vessels of the leg are fully relaxed, but when the perfusion has been in progress for a few minutes signs of a spontaneous recovery of tone appear. The perfusion

pressure has to be raised progressively, frequently to 150 mm. of mercury or more, as the resistance increases, in order to maintain an efficient circulation. A plethysmograph record covering this period shows that a rapid shrinkage of the organ accompanies the fall in venous outflow. Presently outflow and volume become practically constant, without further change of perfusion pressure and this condition may persist with little change for a varying period up to 20 minutes. Sooner or later, even if no injection is made, this spontaneous tone begins to subside and the pressure has to be lowered again to maintain the perfusion within reasonable limits of speed. Fig 3 illustrates this phenomenon, as seen

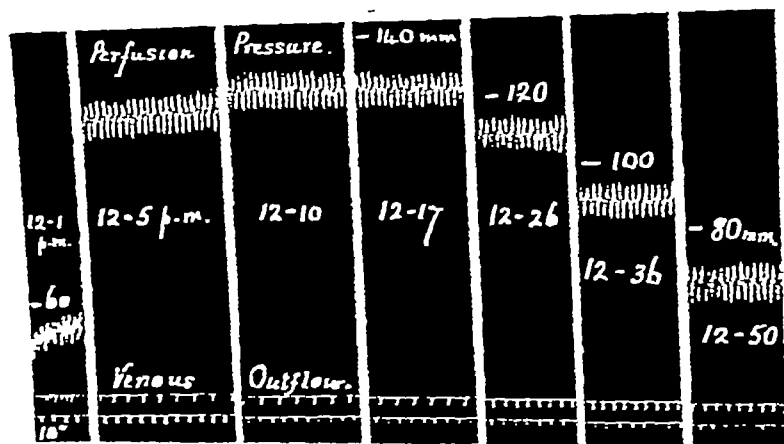


Fig. 3. Acquisition and loss of spontaneous tone by vessels of a cat's legs during the first hour of a perfusion with plain, whipped blood.

in a perfusion of both hind limbs of a cat, made for another purpose. Comparison of the height of perfusion pressure with the rate of outflow at different periods will show the rapid onset and gradual subsidence of the vascular tone. It is during this early period of the perfusion, in which it seems reasonable to regard the tone of the perfused vessels as similar to that which they exhibit in the body even after denervation, that the histamine dilatation can readily be demonstrated. After it has passed, histamine will produce simple vaso-constriction. Further, histamine itself in somewhat larger doses, such as 0.02 mgm., accelerates the disappearance of the spontaneous tone. If histamine, therefore, is given, in more than minimal doses, either too early or too late in the perfusion, the development of this natural tone, and its relaxation by histamine are easily missed altogether and we think it probable that

this circumstance explains the earlier failures to observe it. Figs 1 and 2 *B* are taken from later stages of the same experiments as Figs 1 and 2 *A*. After the effect shown in Fig 2 *A* a dose of 0.02 mgm of histamine was given and produced a prolonged vaso-dilator effect, the vascular tone never being regained. Further injections of histamine produced simple vaso-constriction, as shown in Fig 2 *B*. When once the tone which histamine relaxes has thus been lost, we have never observed its spontaneous return under further perfusion. Thereafter, other vaso-dilators, such as acetyl-choline, continue to produce their normal effects, while histamine as regularly produces vaso-constriction as long as the preparation remains in condition to demonstrate vascular reactions.

Our problem, therefore, was to compare the effect of different vaso-constrictor substances added to the perfusion fluid, not in producing initially a vascular tone which histamine would relax, since this could occur spontaneously, but in restoring such tone when it had finally disappeared.

(b) *Effects of added vaso-constrictor substances* *Adrenaline*. Our experience with this substance uniformly confirmed that of Dale and Richards. Adrenaline has never failed to reproduce such a condition in the vessels that histamine would again exhibit its vaso-dilator effect (Fig 1 *C*), even in larger doses, up to 0.1 mgm. In several experiments adrenaline has succeeded after other vaso-constrictor substances had failed. The concentration necessary has varied with the condition of the preparation. If this is good, as little as 1 part in 20 millions suffices. On occasion a concentration such as 1 in 5 millions has not been adequate, and it has been necessary to raise the strength to 1 in 2 millions, raising the perfusion to 200 mm of mercury or more, in order to obtain an adequate perfusion rate against the high resistance thus produced. Only under two conditions have we known adrenaline to fail, viz when sufficient ergotoxine had been given to paralyse its vaso-constrictor effect, and when, as the result of long perfusion, the tissues had become so oedematous that, when vaso-constriction with adrenaline was produced, further perfusion became impossible.

Pituitary (posterior lobe) extract. It was important to examine the effect of pituitary extract, because it contains the only known potent vaso-constrictor principle, other than adrenaline, occurring naturally in the body, and because Krogh and his co-workers⁽⁹⁾ had shown that this principle had a tonic action on the cutaneous capillaries of the frog. We made five experiments in which the extract was added to the perfusing blood in quantities corresponding to one part of dry, acetone-extracted posterior lobe to 40,000 of blood. In most cases an obvious

vaso-constriction was produced, so that the pressure had to be raised to maintain a good rate of perfusion. In two cases a previous constrictor effect of histamine was converted into the normal, dilator effect. One of these is illustrated in Fig 4

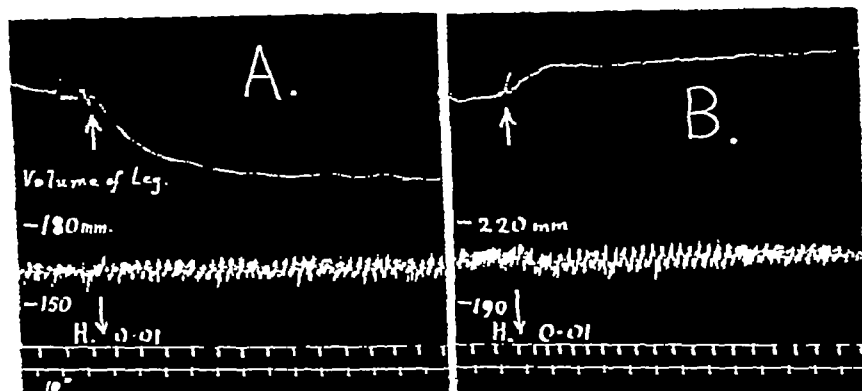


Fig 4. Restoration of dilator response to histamine by adding pituitary extract to perfusing blood.

There can be no doubt, therefore, that the pituitary principle can restore the lost tone to those vessels on which histamine exercises its dilator action. Its effect in this direction cannot, however, be demonstrated with anything like the same ease and regularity as that of adrenaline. In two of the three other experiments, in which pituitary extract completely failed to effect this restoration, adrenaline was subsequently added, with the usual success.

Barium chloride This vaso-constrictor substance was tried in one experiment only. It was added to the perfusing blood so as to produce a concentration of 1 part of BaCl_2 in 10,000. A great increase of peripheral resistance resulted, showing that the barium was exercising a potent vaso-constrictor action. In spite of this the vaso-constrictor effect of histamine was unchanged. The conclusion seemed to be justified that, under the conditions of artificial perfusion, the vascular tone produced by barium did not involve the vessels which histamine dilates.

Ergotamine This alkaloid of ergot, shown by Dale and Spiro (10) to be identical in action with the earlier studied ergotoxine, produces in the spinal animal a general, peripheral vaso-constriction of great intensity and persistence. We observed no vaso-constrictor effect following its injection into the arterial cannula leading blood to the perfused organ.

ments The vaso-dilator effect of histamine was in every case pronounced, and was shown both in the acceleration of the venous outflow and in the increase of volume recorded by the plethysmograph (Fig 7 B) In

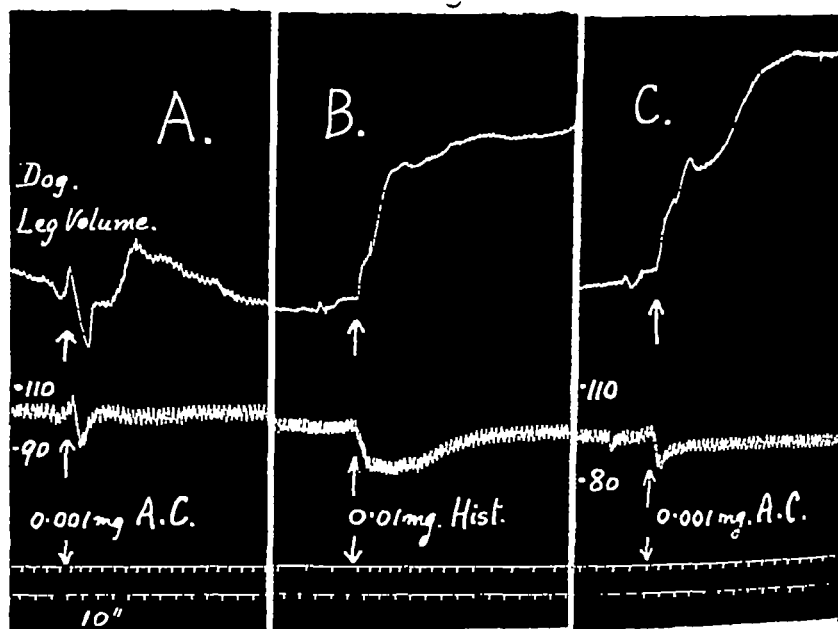


Fig 7 Dilator effects of acetyl choline and histamine on vessels of a dog's leg perfused with blood. C shows improved effect of acetyl choline after histamine.

several ways this vaso-dilator action of histamine on the artificially perfused organ of the dog differs from that seen under like conditions in the cat No special precautions and no choice of a favourable moment appear to be necessary for its observation It appears to be obtainable at any time, and exhibits no special evanescence Nevertheless, if records of experiments in which acetyl-choline and histamine are injected alternately are carefully studied, in the light of what is known of the action of both on the cat's vessels, they are found to have features suggesting that the two actions are not identical in localisation An initial injection of 0.001 mgm of acetyl-choline was found in several cases to produce a surprisingly small and evanescent effect on outflow and on volume (Fig 7 A) If this was followed by an injection of 0.01 mgm of histamine, which from analogy with their respective actions on the cat should be roughly equivalent, the effect was found to

be much more intense and persistent. If now, when outflow and volume were once more steady, a second dose of 0.001 mgm of acetyl-choline was given, the result was conspicuously greater than that produced by the first (Fig 7 C). The sequence suggested that the histamine had weakened a resistance peripheral to the point of action of acetyl-choline, enabling the latter now to produce its full effect.

Though such indications suggested that histamine dilated capillaries in the dog, as in the cat, the regularity with which its vaso-dilator effect could be demonstrated, even after prolonged perfusion and repeated dosage, suggested that the arterioles in the dog were also involved in the action. This suggestion was directly confirmed by perfusion of a preparation made from the dog's superior mesenteric artery with its fine arterial branches. The conditions were similar to those employed for the corresponding preparation made from the cat. Two such experiments were made, and in each case injection of a small dose of histamine caused a small, but definite acceleration of the outflow from the cut ends of the arterioles (Fig 6 B). It will be seen that, even in the absence of added adrenaline, the small arteries of the dog exhibit a tone which histamine relaxes.

On the other hand, strips cut from larger arteries of the dog, such as the iliac or carotid arteries, and suspended in oxygenated Locke's solutions, always responded to histamine by contraction (Fig 8).

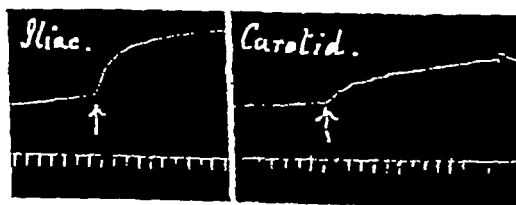


Fig 8 Isolated strips of large arteries of dog. Constrictor effects of histamine (1 in 200,000)

The difference between the action of histamine on the vessels of the dog and the cat would, therefore, seem to be of this kind, that the change from constrictor to dilator action takes place at different levels in the vascular branching. In the dog it occurs so early that arteries still macroscopically recognisable as such are already involved in the dilatation, whereas in the cat the change occurs at some more peripheral point.

(3) *Perfusion of the monkey's leg*

We have had the opportunity of carrying out artificial perfusions on the legs of only two monkeys, but the results enable us to state that the effects of histamine on the vessels of this species resemble those in the dog rather than those in the cat. The perfusions were carried out with the plain whipped blood of the monkey from which the leg was obtained, and histamine regularly produced a conspicuous vaso-dilator effect (Fig 9), which showed no signs of disappearing or changing to vaso-constriction with repeated injections or continued perfusion. The evidence strongly suggests that in the monkey, and therefore probably in man, the dilator effect of histamine is not restricted to the capillaries, but extends to the arterioles, as in the dog.

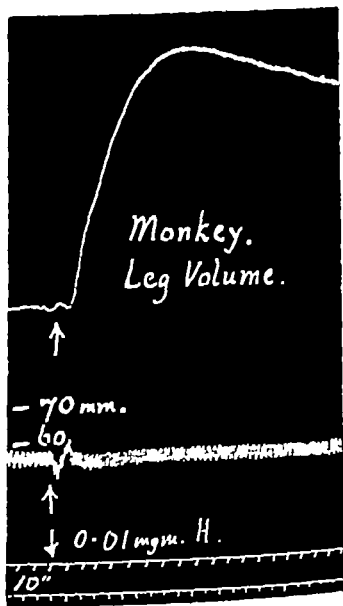


Fig 9 Effects of histamine on vessels of monkey's leg perfused with blood.

(4) *Discussion*

The experiments above described have shown that in species, other than the cat, which show a vaso-dilator response to histamine under conditions of natural circulation, this response can be demonstrated under artificial perfusion with the same ease and certainty as that of other vaso-dilator agents. The suggestion that the depressor effect of histamine was due to effects of other types would presumably not have arisen, but for the accident that the cat, rather than the dog or the monkey, was chosen for the early experiments on the nature of its action. Even in the perfused vessels of the cat, under conditions as nearly normal as they can be made, our experiments show that the dilator response to histamine is not really wanting, though very easily lost. When we find that the cat also differs from the dog, in that the finest separable arteries are constricted by histamine in the cat and dilated in the dog, it is natural to associate the two points of difference, and to suppose that the evanescence of the dilator effect in the cat is due to its incidence

mainly on the capillary vessels. This is substantially the conclusion reached by Dale and Richards, our evidence only differs from theirs in showing that the dilator response of the cat's capillaries is not unobtainable, as they supposed, if the perfusion fluid does not contain both red corpuscles and added adrenaline, but only peculiarly short-lived.

This conclusion raises the question as to the nature of the agent in the shed blood which, for a time, restores a tone to those vessels in the perfused organ of a cat which histamine relaxes. We have suggested that this tone is comparable to that which the vessels acquire in the body shortly after denervation. It would be natural in the latter case to suggest that the vaso-constrictor hormones, adrenaline and the pituitary pressor principle, are the agents concerned. If that suggestion is accepted, it is equally natural to suppose that traces of these hormones in the shed blood are responsible for the temporary tone which appears in the early stage of a perfusion. Of the two, adrenaline appears, on the evidence before us, to be the one which is likely to be the more important factor. Krogh, indeed, has shown that the capillaries of certain tissues in the frog, though only of some, are highly responsive to the tonic action of the pituitary principle, and several observers, among them Sacks (11), have described a peculiar sensitiveness of the skin capillaries in man to this hormone. On the other hand, our experience with the perfused organ of the cat suggests that adrenaline has, in this species, a unique effectiveness in reviving the tone of the vessels dilating with histamine, when once this has been lost. Pituitary extract will sometimes effect this revival, but only in such degree as to suggest rather a potentiation of the effect of a remnant trace of adrenaline, than a direct and specific action of its own. The evanescence of the naturally acquired tone, and its regular and prompt return when adrenaline is added, accord best with the view that this same, unstable substance is responsible throughout for its appearance.

There are, indeed, difficulties in the way of attributing to adrenaline this chief rôle in the maintenance of tone in the denervated vessels of the living animal, whether arteries or capillaries. Chief among these is the fact, already discussed by Dale and Richards, that adrenaline itself, injected into the circulation in very small doses, produces a vasodilator reaction of such vessels, very similar to that produced by a minute dose of histamine. The point can better be discussed later, in connection with experiments on the whole animal, which further illustrate the intimate nature of the antagonism between adrenaline

and histamine in their vascular effects. Here we may note, in passing, that we have found it impossible to demonstrate a vaso-dilator effect of adrenaline on the perfused leg of the cat or dog, however high the initial tone of the vessels, however pronounced their vaso-dilatation in response to histamine, and however small the dose of adrenaline injected. The latter, if large enough to produce any perceptible effect, has always produced an uncomplicated vaso-constriction. Only when ergotoxine (ergotamine) had been previously injected could we obtain a vaso-dilator effect of adrenaline on the perfused organ.

Another point calling for comment is the rôle of the red corpuscles in facilitating the demonstration of the dilator effect of histamine on the perfused organ. Dale and Richards had found that red corpuscles must be present and that adrenaline must be added. While our experiments do not strictly confirm this conclusion, showing that either gum solution containing adrenaline or plain blood will often suffice, they do show that the presence of red corpuscles has an effect definitely favourable to the vaso-dilator response to histamine. When they are present, adrenaline can always restore it easily when it has disappeared, with gum solution and adrenaline the effect may be shown early in the perfusion, but, when once it has disappeared, further doses of adrenaline apparently cannot revive it. Dale and Richards attributed the favouring action of red corpuscles to more efficient oxygenation of the tissues, and this may, indeed, be a factor in their influence. It seems probable to us, however, that the explanation is rather to be sought in mechanical considerations, arising from the observations of Krogh, which have since become available. Krogh showed that in a large part of the capillary vessels of the body, and particularly in those of the resting skeletal muscles, a condition of tone normally exists, such that the lumina, even of the small proportion open at all, are so narrow that the elastic red corpuscles must be deformed to pass through them. Under such conditions, even though the total area of the open capillary path may be large in relation to that of the arteries, it appears certain that a material part of the peripheral resistance must be in the capillaries, and that even a slight relaxation of these will produce large effects on the venous outflow and the volume of the organ. Even a moderate, normal capillary tone would apparently suffice to produce these phenomena when corpuscles are present. In their absence, on the other hand, the total area of the capillaries may well be so great that no important part of the peripheral resistance is produced in them, unless their tone is abnormally high, so that few are open at all.

The fact that the small arteries are dilated by histamine in the dog, and probably in the monkey, makes it difficult to obtain evidence from perfusion experiments as to its action on the capillaries in these species. Evidence of other kinds is not wanting, however. Dale and Laidlaw⁽¹²⁾ found that histamine in larger doses produced a permanent, shock-like collapse of the circulation, with oligæmia and rise of corpuscular content, in the dog as in the cat. Such an effect has not been demonstrated with any simple arterial dilator, and it is unlikely to be produced by different mechanisms in the two species. More direct evidence is afforded by the observations of Abel and Geiling⁽¹³⁾ and Geiling and Kolls⁽¹⁴⁾, who described the vivid general erythema produced in a white-skinned dog, when a small dose of histamine or the similarly acting albumose was given intravenously, and observed and photographed with the microscope, in the latter case, the dilatation of capillaries and venules causing it. The observations of Sollmann and Pilcher⁽¹⁵⁾, and especially the more recent work of Lewis and Grant⁽¹⁶⁾, have shown that histamine directly dilates the skin capillaries in man. In the frog Killian⁽¹⁷⁾ has shown, by direct microscopic observation and photography of the vessels of the tongue, that the dilator action of histamine extends to arterioles and capillaries. In the fowl histamine has a depressor action, superficially quite similar to that which it shows in other species, and this can safely be assumed to be vaso-dilator in type. Among the vertebrates hitherto examined, the rodents (rabbit and guinea-pig) appear to form a conspicuous exception. There is no clear evidence that histamine exerts a vaso-dilator action on any part of the vascular system in these. A constrictor effect on systemic and pulmonary arterioles is easily detected, and the action on the pulmonary vessels may be so severe as to cause acute dilatation of the right chambers of the heart—an effect seen also in the cat as a passing phase in the action of large doses. But the vaso-dilator effect cannot be detected in the rodents, at any rate under conditions which are suited to its demonstration in other species.

So far as its effects on the arterial side of the circulation are concerned, the action of histamine may be pictured, in general terms, as consisting of a constrictor effect on the more central part of the vascular tree (i.e. on the part nearer the heart), changing to a dilator effect on more peripheral branches. The differences noted between its action in different species may then be regarded as due to differences in the level at which this change occurs. In the dog, and probably in the monkey, it occurs before the smallest macroscopic arteries are reached, in the cat it occurs more peripherally, so that the dilator effect is mainly, if

not entirely, exercised on the capillaries, in the rodents it is pushed so far to the periphery as to escape detection, and possibly does not exist at all

PART II EFFECTS OBSERVED WITH NATURAL CIRCULATION

We made a number of experiments on cats anaesthetised with ether, or decapitated under preliminary anaesthesia, in order to examine the possibility of restoring a failing vaso-dilator response to histamine, by injecting into the naturally circulating blood the vaso-constrictor substances which we had previously tested in this direction on the artificially perfused organ. The interpretation of the results proved to be so complicated that we do not propose to discuss them at present. The experiments led us, however, to the study of certain effects produced by histamine and by adrenaline, which seems to throw important light on the physiological antagonism between their respective types of action.

1 *Secondary pressor effect of histamine* When the general arterial pressure of a spinal preparation has fallen to a low level, through failing efficiency of the spinal vaso-motor centres, injection of a small dose of histamine (such as 0.01 mgm) still causes a definite, though often relatively trivial, further fall of the arterial pressure. This fall, however, is frequently followed by a secondary rise of the pressure, which may be extensive, and is habitually more conspicuous than the preliminary fall. Attention has been specially directed to this secondary phase of the action of histamine, under condition of low arterial pressure, by Hogben, Schlapp and Macdonald (18), who encountered it as a complication in determining the specific pressor action of certain pituitary extracts. We found that its relative prominence was exaggerated, if we restricted the vascular capacity of the spinal preparation by removing the stomach and intestine and excluding the liver from circulation. The phenomenon, as it was habitually observed in such preparations, is illustrated in Figs 10 A and 11 A. In considering the nature of this pressor effect, we naturally had in mind the double action of histamine, as shown on the vessels of the perfused organ. Histamine has been shown to have a constrictor action on the small arteries of the cat, in addition to its more peripheral dilator action. Nothing in our experience with perfused organs, however, corresponded to this sequence of a dilator followed by a very powerful, delayed constrictor action. Inspection of the manometer, moreover, during the production of such records, revealed suggestive features, which are not clearly visible in the tracing on a slowly moving

surface. The initial fall of pressure was accompanied by such moderate acceleration of the heart-beat as normally accompanies the depressor

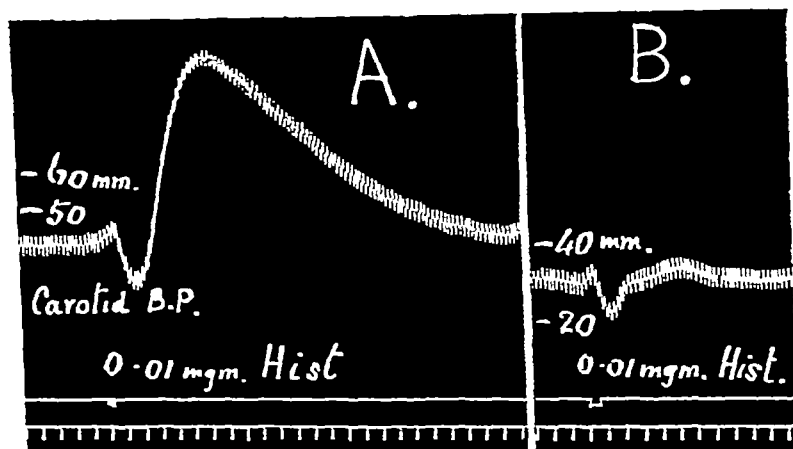


Fig 10 Arterial pressure of spinal cat to show secondary pressor effect following injection of histamine, (A) before, (B) after extirpating suprarenal glands

action of a small dose of histamine, and this passed off as the pressure curve turned upwards again. Soon after the turn, however, a second, much more pronounced acceleration of the heart began abruptly, and continued as the pressure rose rapidly to the secondary peak, subsiding during the return to the normal. This suggested the sudden entry into the circulation of an agent other than histamine. Inspection of plethysmograph records from a denervated limb reinforced this suggestion. During the primary fall of pressure, following injection of a small dose of histamine when the arterial pressure was low, the limb might show a small expansion or a small shrinkage of volume, concurrently with the secondary pressor phase the limb volume showed a secondary, much more pronounced and rapid shrinkage.

The fact, clearly demonstrated by Kellaway and Cowell (19), that a small intravenous injection of histamine is regularly followed by a brief acceleration of secretion from the suprarenal glands, suggested that the secondary pressor effect might be due to such a small gush of adrenaline, and not a direct effect of the histamine itself. The suggestion was supported by the observation that, when successive small injections of histamine were made into the vein of a spinal cat, at fairly short intervals, the secondary pressor effect became gradually smaller, but recovered when the preparation was left for a longer time without injection. The

possibility was tested in two ways. In several experiments, in which the pressor effect was initially well marked (Fig 10 *A*), the suprarenal glands were extirpated, with the result that subsequent injections of histamine produced small depressor effects, with practically no secondary pressor phase (Fig 10 *B*). In another case, in which the pressor effect was unusually pronounced (Fig 11 *A*), ergotamine was injected in

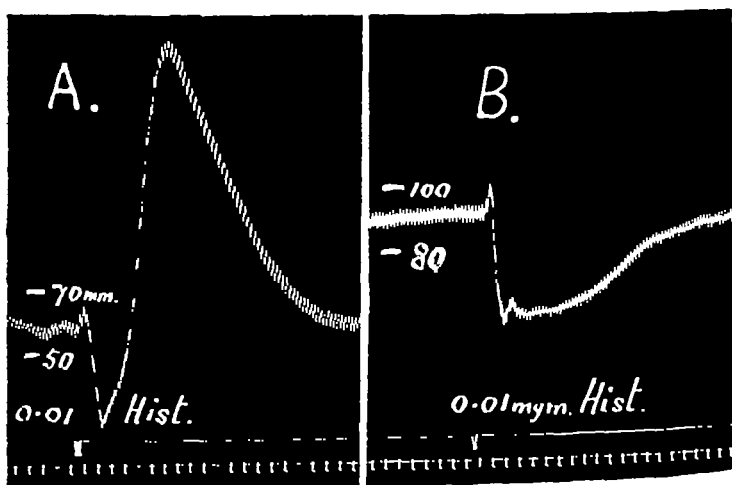


Fig 11 Similar to Fig 10, (*A*) before, (*B*) after ergotamine.

sufficient dose to reverse the pressor action of adrenaline, an injection of which now caused a simple fall of arterial pressure. Another dose of histamine was then injected, and in place of the pressor phase a secondary depressor action was produced, much more prolonged than that primarily caused by the histamine, and beginning just as the latter had passed its maximum (Fig 11 *B*). This secondary depressor action was accompanied by acceleration of the heart-beat, such as adrenaline itself produces, under the same conditions.

This evidence seems to put it beyond doubt that the delayed pressor action, which follows the injection of histamine under these conditions of low arterial pressure, is not a direct histamine effect at all, but is due to the suddenly accelerated output of adrenaline from the suprarenal glands. We believe the effect to be a really specific one. Though it has been shown that the adrenaline content of the suprarenal blood is somewhat increased by any influence which lowers the general arterial pressure, other depressor agents do not cause such an accelerated output as is represented by this pressor after-effect of histamine. An equi-

pressor dose of acetyl-choline for example, given under identical conditions produces nothing comparable to it

2 *The depressor effect of adrenaline* The fact has long been known that a very small dose of adrenaline, injected intravenously into an anaesthetised cat or dog with good vascular tone will produce a predominantly depressor, vaso-dilator effect the more familiar pressor effect of adrenaline forming under such conditions, a trivial preliminary phase of the action. The nature of this effect was fully considered by Dale and Richards who mentioned the earlier literature dealing with it. They were impressed by the similarity of this vaso-dilator action of adrenaline, in its distribution and in the conditions favouring its appearance to that of histamine and concluded that it was probably located on the same part of the peripheral vessels i.e. on the capillaries in the cat. This conclusion produced a difficulty of conception which they were unable adequately to resolve. They were led by other considerations to the view, which our own evidence supports, that adrenaline is probably the most important factor, in the absence of nervous control, in maintaining in the cat the capillary tone which histamine inhibits. The vaso-dilator action of adrenaline being similar to that of histamine we are then faced with the necessity of supposing that a steady content of adrenaline would maintain a tone which a small sudden, extra injection of the same substance would inhibit. We tested this not very easy supposition by giving a very slow, steady infusion of adrenaline into the vein of a cat under conditions in which a small sudden injection produced the vaso-dilator depressor effect. The result is shown in Fig. 12. It was clear that adrenaline artificially introduced at a slow steady rate into the venous

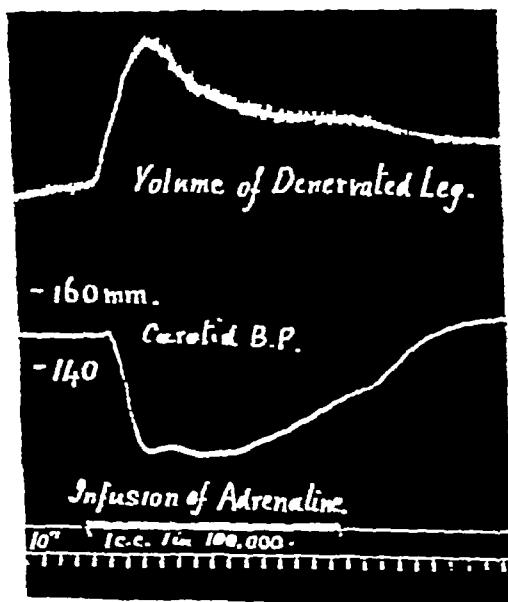


Fig. 12. Arterial pressure and volume of denervated leg of cat under ether. Very slow infusion of adrenaline into jugular vein.

blood, is able to produce a protracted, though limited fall in the peripheral resistance. The difficulty of attributing to adrenaline a steadily maintained tone, which a small, sudden dose will relax, appeared to be accentuated.

The evidence presented in the preceding section shows that the effect of histamine, injected into the circulation of the living animal, is complicated by a secondary action, not represented at all in its effects on the perfused organ, and due to the response of the suprarenal glands. Now the vaso-dilator effect of small doses of adrenaline, as seen in records of arterial pressure and denervated limb volume in the whole animal, is also a second phase of its action, following with a relatively long latent period a small, primary pressor effect, and it is similarly not represented by any demonstrable action of adrenaline on the perfused organ. If it could be shown that it was due to the output, in response to the adrenaline injection, of a small quantity of a substance having an action like that of histamine, the difficulties encountered in interpreting its meaning would disappear. The identity of the species showing vaso-dilator effects with histamine and with adrenaline, the correspondence of the conditions intensifying these two effects, and the failure to produce the vaso-dilator action of adrenaline on an isolated organ, would all be adequately explained.

If such an output of a histamine-like principle was concerned in this effect, there was no reason to expect that it would issue from a special glandular organ, as adrenaline does. On the contrary, evidence has accumulated during recent years, in specially convincing form in the work of Lewis and Grant(16), in favour of the view that such a principle is constantly being formed in all the tissues, its production being accelerated by slight injury of any kind, and that it plays an important part in regulating the capillary circulation. If its production, in response to injection of adrenaline, had this general distribution, there would be no obvious method of obtaining evidence of it. It occurred to us, however, that there is one organ, the lungs, through which adrenaline, given by the ordinary method of intravenous injection, has to pass, before it reaches the systemic circulation. In the lungs, moreover, adrenaline in these minute doses has never been shown to have any vaso-constrictor action. If any part of the depressor, vaso-dilator effect on the peripheral, systemic vessels should be due to a histamine-like substance shed into the blood from the lungs, in response to the passage of the adrenaline through their vessels, we should expect that the depressor effect would be larger, and that its latent period would be

shorter, when the adrenaline is injected into a vein, than when it is injected, in equal dose, directly into the arterial stream. We have made the experiment six times, on cats under ether, and the result has uniformly confirmed this expectation

Arterial pressure was in all cases recorded from a carotid artery. For the arterial injections we chose the left subclavian artery, since its separate origin, beyond that of the innominate ensured that an injection made through its central stump into the aorta would all be carried caudally in the blood of the descending aorta. It was necessary only to tie off the branches arising from it between the cannula and its aortic origin, to prevent any part of the injection becoming side-tracked and failing to enter the aortic stream. We found it not difficult to tie off the thyroid axis, vertebral and internal mammary arteries by dissection behind the pleura. A bull-dog clamp was then applied and a cannula tied centrally into the subclavian artery beyond it. A similar cannula was tied into the external or the internal jugular vein, just above its entrance into the superior vena cava. The cannulae were filled with adrenaline solution of 1 in 100,000, and a small syringe containing the requisite small dose of the same solution was attached to one or the other. The clamp on artery or vein was then opened and the syringe rapidly emptied into the vessel. In one experiment we recorded also the volume of a hind leg, denervated a week previously by aseptic section of the sciatic and anterior crural nerves.

The results were in all cases the same. A small dose (0.002-0.004 mgm.) of adrenaline, injected into the vein, produced a small, sharp rise of arterial pressure, apparently due to acceleration of the heart, which was immediately succeeded by the characteristic depressor action. The same dose, injected into the aorta, usually caused a slighter vaso-constrictor rise of arterial pressure, which was sometimes so small as to be barely perceptible, but when visible was much more persistent than that caused by the intravenous injection. When this rise had subsided, there followed a depressor effect conspicuously smaller than that forming the main phase of the effect following intravenous injection, and sometimes so small as to be hardly detectable. A characteristic sequence of such effects is shown in Fig. 13 A, B and C.

Several possible explanations of this contrast had to be considered. We had to make sure, in the first place, that our arterial injections really delivered the full dose into the main arterial stream. This was controlled by injecting small doses of histamine through the same arterial and venous cannulae. The depressor effects of these were indistinguishable

(Figs 13 and 14 *D* and *E*), except by their latent periods, as presently to be mentioned. Then it might be suggested that the venous injection alone gave the adrenaline early access to the coronary vessels, as shown

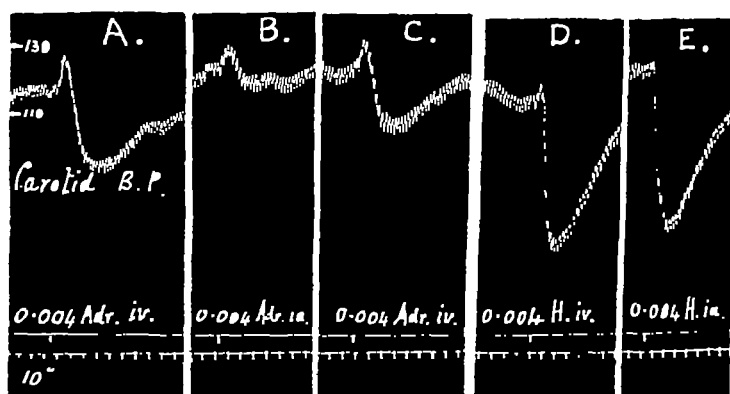


Fig 13 Depressor effects of small doses of adrenaline in cat under ether, given intravenously (*A* and *C*) and intra arterially (*B*). *D* and *E* show similar injections of histamine for comparison.

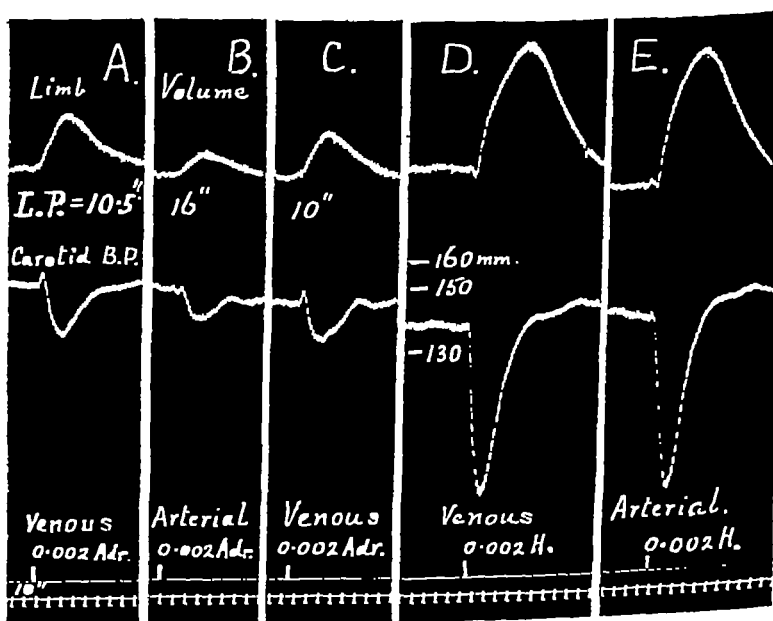


Fig 14 Similar to Fig 13, with plethysmograph record of volume of denervated limb

by the accelerator effect on the heart, and that dilatation of the coronary vessels might cause the fall of carotid pressure, or, again, that the small dose of adrenaline, in passing through the lungs, produced sufficient additional resistance in their vessels to reduce the inflow, and therefore the output, on the left side of the heart. Both these explanations on other grounds not very probable, appear to be completely excluded by the fact that the falls of arterial pressure are accompanied by corresponding dilatations of the denervated leg, as shown in Fig 14 *A, B* and *C*, from which it can be seen that the volume-record displays an entirely similar contrast between the effects of arterial and venous injections of the same small dose of adrenaline.

The depressor effect following the arterial injection is not only smaller, the latent period of its onset is conspicuously longer. It is not easy to get exact measurements of the latent period of a depressor immediately following a pressor effect, owing to the difficulty of separating subsidence of the former from onset of the latter, but the difference is so conspicuous that great accuracy is not needed. In one case, in which measurements were made from a record on a rapidly travelling surface, the estimate for the latent period with venous injection was 9 seconds, with arterial injection 13 seconds, difference 4 seconds. On the volume record the small, preliminary pressor phase has usually no perceptible effect, and the beginning of the expansion can be detected with reasonable precision. One of us, making the injection, signalled to an assistant with a stop-watch as the piston was pressed home. The other, watching the volume record, signalled the onset of the dilatation. The following were the times recorded in seconds by this method.

Dose of adrenaline	Venous injection	Arterial injection	Difference
0.004 mgm	7	14	7
		12	5
0.002 mgm.	11.5	17.5	6
	10	16	6

Average 6 seconds

The differences are, in each case, between the latencies of consecutive injections. It will be seen that the latent periods with the smaller dose are uniformly longer than with the larger, but that the average differences between the arterial and venous delays are the same. The method of measurement, though crude, was sufficiently accurate, and the difference observed was widely outside its possible error.

We may safely conclude therefore, that the latent period of the depressor effect following arterial injection is longer by some 4-6 seconds

than that of the effect following the ordinary intravenous injection. When histamine, on the contrary, was injected by the same methods, we found, in confirmation of earlier observations (cf. Dale and Richards) that the depressor effect, and the increase of limb volume, began a second or two earlier with arterial than with venous injection, as would be expected of an effect due to direct, peripheral vaso-dilator action. The conclusion seems to be inevitable that the vaso-dilator effect produced by an injection of adrenaline is not due to an action of this type, but is secondary to the liberation, in response to the adrenaline injected, of a vaso-dilator substance. We should expect an effect of this secondary kind to have a relatively long latency in any case. On the supposition that the place of origin of the dilator substance is in the lungs, we should expect that a large part of an arterially injected dose of adrenaline would disappear before reaching them, and that the time taken to travel round the major circulation would involve an additional latency of some 4-6 seconds. The results observed confirm all these expectations.

3 *Discussion* The results of these experiments strengthen the suggestion, which has frequently been made, that there is a special physiological antagonism between adrenaline, on the one hand, and a capillary-dilator principle, on the other hand, which closely resembles histamine in its action. Dale and Richards pointed out that the liberation of such a principle, as the result of metabolic activity, would provide a perfect fine adjustment of the capillary circulation to the needs of the tissues, and suggested that an important function of the normal output of adrenaline, from the suprarenal glands, might be so to balance and antagonise this dilator action as to maintain or restore a normal capillary tone. This conception seems to be supported by the evidence that each of these substances, injected in small dose, causes an accelerated output of its antagonist. Under appropriate conditions, a minute dose of histamine produces an effect in which the action of adrenaline is more prominent than that of the histamine itself, while a small dose of adrenaline, injected under the alternative conditions, produces an effect which is mainly of the histamine type, and apparently due to the output of a histamine-like principle. It is hardly likely that this natural output of each antagonist, in response to the sudden appearance in circulation of an excess of the other, occurs only under the conditions favouring its detection. It is, of course, obvious that low vascular tone will accentuate the secondary (adrenaline) effect of histamine in comparison with its direct depressor action, while high vascular tone will similarly accentuate the secondary (histamine) effect

following the injection of adrenaline, in relation to, and at the expense of, its direct vaso-constrictor action. We suppose however that under conditions rendering these secondary effects less obvious the effect of a dose of either of these substances is really modified and rendered more evanescent by a compensatory output of its antagonist. The direct effect of either substance on the arterial pressure, even under conditions not specially favouring the effect of its antagonist, can, indeed, be observed, in many cases to be followed by a swing of the pressure in the opposite direction, before equilibrium at the original level is re established. That such a compensation is normally in action is further strongly suggested by the observation, already mentioned of the greatly accentuated effect of a small dose of histamine when the adrenals or only their medullary portions, have been extirpated.

We have presented evidence which seems to point to the lungs in particular as a source of a histamine-like antagonist to adrenaline. It should be made clear, however, that our evidence only deals with the response to a sudden artificial injection of adrenaline, by output of a depressor substance in such quantity as to over-compensate the direct adrenaline effect. The facts do not warrant the suggestion that the lungs are the only or even the principal place of origin of such a substance under physiological conditions. As to the part they might play in relation to the passage through them, on its way to the major circulation, of adrenaline issuing at normal rate from the suprarenal gland, we have no material even for conjecture.

There are many points in this connection which can only be elucidated by further experiment. We must content ourselves, for the present, with the presentation of evidence which reinforces the suggestion that action of the histamine type is no mere pharmacological curiosity, but one of genuine physiological importance in providing, at least in many species, one side of the balanced chemical control of capillary tone, of which the other is provided chiefly by the natural secretion of adrenaline.

SUMMARY

- 1 The vessels of a cat's limb, artificially perfused with blood, are relaxed by histamine. The tone favouring this reaction is evanescent but can regularly be restored by a trace of adrenaline, and occasionally by pituitary extract.
- 2 The vessels of the limb of a monkey or dog, artificially perfused, are relaxed by histamine, and the reaction can be obtained repeatedly.
- 3 The perfused arterioles of the cat are constricted, those of the dog

than that of the effect following the ordinary intravenous injection. When histamine, on the contrary, was injected by the same methods, we found, in confirmation of earlier observations (cf Dale and Richards) that the depressor effect, and the increase of limb volume, began a second or two earlier with arterial than with venous injection, as would be expected of an effect due to direct, peripheral vaso-dilator action. The conclusion seems to be inevitable that the vaso-dilator effect produced by an injection of adrenaline is not due to an action of this type, but is secondary to the liberation, in response to the adrenaline injected, of a vaso-dilator substance. We should expect an effect of this secondary kind to have a relatively long latency in any case. On the supposition that the place of origin of the dilator substance is in the lungs, we should expect that a large part of an arterially injected dose of adrenaline would disappear before reaching them, and that the time taken to travel round the major circulation would involve an additional latency of some 4-6 seconds. The results observed confirm all these expectations.

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THE CENTRAL AND REFLEX REGULATION OF THE HEART RATE BY G V ANREP AND H N SEGALL

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THE adaptation of the heart beat to changes in circulatory conditions is attained by two separate mechanisms (a) by the adaptation of those parts of the central nervous system which regulate the rate and the strength of the cardiac contraction, and (b) by the adaptation of the heart muscle itself. While the rate of a denervated heart remains unaffected by changes in the circulatory conditions and is mainly determined by the temperature of the blood the rate of the heart in the whole body is influenced in addition by a great number of changes in the mechanical conditions of the circulation. This difference to a large extent is explained by the presence of the extra-cardiac mechanism of adaptation which is lacking in the heart-lung preparation. The use of the whole animal for the study of this mechanism is not entirely suitable because it is difficult to control independently the cerebral and the systemic circulation and to decide whether changes in cardiac activity are of central, reflex, or peripheral origin. The different methods of interarterial and arteriovenous crossed circulation which have been introduced to overcome these difficulties do not materially improve the control over the circulation. The newer methods of crossed circulation as used by Anrep and Dalva⁽¹⁾, Anrep and Starling⁽²⁾ and Hermans⁽³⁾ though they place the cerebral circulation under a better control yet leave the systemic circulation still uncontrolled. The experiments to be described were performed with a new technique which allows an independent control over the circulation in the brain and in the heart. Briefly this technique consists in establishing an *innervated* heart-lung preparation in contrast to the denervated preparations of Martin⁽⁴⁾, Hering⁽⁵⁾ and Starling⁽⁶⁾.

The innervated heart-lung preparation Two dogs are used for each experiment. From one dog blood is collected, whilst the second dog is injected with morphia, anaesthetised with chloralose (0.075 gm. per kilo), and bled from the femoral artery about one-quarter of its total blood volume. Artificial respiration with room air is started, and the

relaxed by histamine. It is suggested that the predominant incidence of the histamine dilator action on the capillaries is peculiar to the cat.

4 There is evidence that a secondary pressor effect of histamine is due to accelerated output of adrenaline, and that the depressor effect of small doses of adrenaline, seen in the anaesthetised cat or dog, is also a secondary effect, due to liberation of a histamine-like principle.

5 The bearing of these observations on the chemical control of capillary tone is discussed.

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THE CENTRAL AND REFLEX REGULATION OF THE HEART RATE BY G V ANREP AND H N SEGALL

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THE adaptation of the heart beat to changes in circulatory conditions is attained by two separate mechanisms (a) by the adaptation of those parts of the central nervous system which regulate the rate and the strength of the cardiac contraction, and (b) by the adaptation of the heart muscle itself. While the rate of a denervated heart remains unaffected by changes in the circulatory conditions and is mainly determined by the temperature of the blood, the rate of the heart in the whole body is influenced in addition by a great number of changes in the mechanical conditions of the circulation. This difference to a large extent is explained by the presence of the extra-cardiac mechanism of adaptation which is lacking in the heart-lung preparation. The use of the whole animal for the study of this mechanism is not entirely suitable, because it is difficult to control independently the cerebral and the systemic circulation and to decide whether changes in cardiac activity are of central, reflex, or peripheral origin. The different methods of interarterial and arteriovenous crossed circulation which have been introduced to overcome these difficulties do not materially improve the control over the circulation. The newer methods of crossed circulation as used by Anrep and Daly⁽¹⁾, Anrep and Starling⁽²⁾ and Heymans⁽³⁾, though they place the cerebral circulation under a better control yet leave the systemic circulation still uncontrolled. The experiments to be described were performed with a new technique which allows an independent control over the circulation in the brain and in the heart. Briefly this technique consists in establishing an *innervated* heart-lung preparation in contrast to the denervated preparations of Martin⁽⁴⁾, Hering⁽⁵⁾ and Starling⁽⁶⁾.

The innervated heart-lung preparation. Two dogs are used for each experiment. From one dog blood is collected, whilst the second dog is injected with morphia, anaesthetised with chloralose (0.075 grm per kilo), and bled from the femoral artery about one-quarter of its total blood volume. Artificial respiration with room air is started, and the

chest is widely opened after a mid-sternal incision. The internal mammary blood vessels on both sides are now cut between ligatures, the left subclavian artery close to its origin from the aorta and the right subclavian artery just beyond the origin of the right vertebrate are tied off. The azygos vein is ligatured in two places, close to its entry into the superior vena cava and about 2 or 3 cm below this point so as to separate entirely the upper intercostal veins from the lower portion of the azygos vein. It is advisable also to ligature the right subclavian vein at its entry into the superior vena cava.

The following blood vessels are then prepared for the insertion of cannulae: the aorta just beyond the origin of the left subclavian artery, the inferior and superior venae cavae, the brachiocephalic artery. One ligature for the brachiocephalic artery is placed intra-pericardially and the other just extra-pericardially.

The apparatus consists of Starling's heart-lung apparatus, together with two small rubber pumps which are rhythmically compressed by adjustable eccentrics and which are provided with valves permitting the flow of blood only in one direction. The aorta and the inferior vena cava are connected to the heart-lung apparatus, while the head is perfused through the brachiocephalic artery by means of one of the pumps. The blood issuing from the superior vena cava is returned through the second pump into the reservoir of the heart-lung apparatus. As can be seen from Fig. 1 the head circulation is maintained in this preparation altogether independently of that in the heart and lungs. Thus there results a heart-lung preparation which retains all its nervous connections intact. The two independent circulations are supplied with blood from a common reservoir and therefore do not differ with regard to the composition of the inflowing blood.

The separation of the thoracic circulation from the cerebral makes it possible to vary the mechanical conditions in each independently. On its way to the brain the blood passes through a separate warming spiral so that the temperature can be varied independently. The circulation in the head in our experiments is not supplied with an artificial compensator and it is therefore possible to observe vasomotor changes in the perfused head. In the absence of the compensator the blood pressure in the cerebral circulation has to be adjusted by altering the stroke of the perfusion pump.

The sequence and mode of introduction of the four cannulae are as follows:

1. The inferior vena cava is clamped and the heart is allowed partially to empty itself. The lower ligature on the aorta is then tied and the aortic cannula introduced. A side

branch of this cannula is opened and immediately afterwards the vena cava is released. The blood which now flows from the cannula is collected and defibrinated, care being taken not

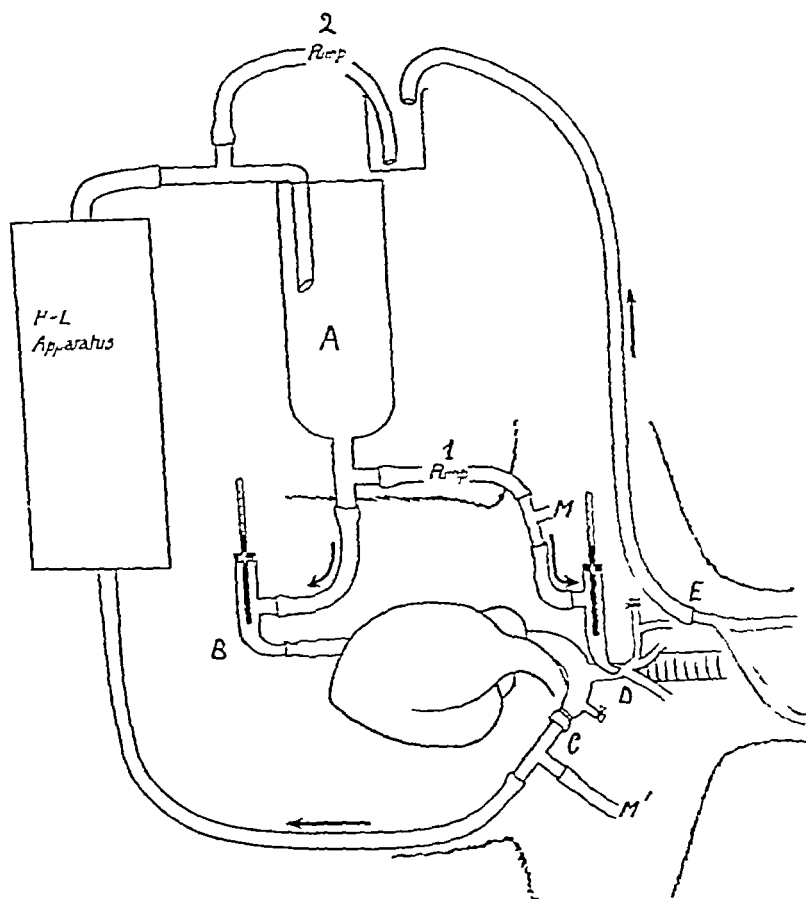


Fig. 1. Diagram of the innervated heart-lung preparation. The heart lung apparatus contains the warming spirals and the artificial resistance. The heart lung circuit consists of the venous reservoir *A* from which the blood flows through cannula *B* into the inferior vena cava, the blood leaves the heart from the aorta through cannula *C*. The cerebral circulation is maintained by pump 1, which drives the blood into the brachiocephalic artery *D*. The blood flowing from the head through the superior vena cava *E* is collected into a beaker and then returned by pump 2 into the venous reservoir. The beaker is kept at the level of the head.

to bleed too much, but to leave enough blood to supply the heart and brain. The side branch of the cannula is then closed and the abdomen compressed so as to press the remainder of the animal's own blood into the heart when the lower ligature on the inferior vena cava is tied.

2 Meanwhile the venous reservoir of the heart-lung apparatus is filled with defibrinated blood to which is added about 0.02 grm. of heparin, in order to prevent the clotting of the blood which remains in the animal. After introduction of the cannula into the inferior vena cava the heart, lungs and brain are perfused. The artificial resistance in the heart-lung apparatus is kept high from the very beginning in order to ensure a good perfusion of the brain and of the coronary arteries in this stage of the experiment.

3 The third cannula to be introduced is that into the superior vena cava draining the brain. The tube connected to the cannula (which is clamped during the introduction) dips into a beaker from which the blood is sucked back into the venous reservoir by one of the two rubber pumps.

4 The last cannula to be introduced is the one into the brachiocephalic artery. All the system of tubes connecting this cannula with the apparatus are filled with blood. The cannula is quickly introduced into the artery, the clip on the superior vena cava is removed, and the circulation through the brain is started, the circulation through the heart and lungs being now separated from that of the brain. The only occasion when the brain is not supplied with blood is during the introduction of the arterial cannula, and this stage is passed through as quickly as possible.

In cases when this operation was unduly prolonged the brain was found to be in a state of diminished activity for a considerable period of time. The conjunctival reflex as a rule disappears even with a rapid introduction of the cannulae, but it quickly returns and remains active as long as cerebral circulation is maintained. The reflexes of deglutition and salivary secretion, and the respiratory efforts continue as before. The artificial respiration is now changed to a mixture containing 4.0 to 4.8 p.c. CO_2 and not less than 60 p.c. of oxygen. This percentage of CO_2 ensures that the high pulmonary ventilation does not render the tissues acapnic, and the high tension of oxygen serves to minimise the effect of the blood issuing from the vena cava superior, reducing the oxygen saturation of the mixed blood in the common reservoir.

The inverse relation between arterial blood-pressure and heart rate which was first demonstrated by Marey (7) has received different explanations. Bernstein (8), François-Frank (9), Biedl and Reiner (10) and Gerhardt (11) believe it to be of a purely central vagal origin. Kochmann (12) regards it as a reflex through the vagus centre, but finds that the centre itself is insensitive to changes in blood-pressure. Filehne and Biberfeld (13) state that the slowing of the heart rate depends entirely on the rise of intracranial pressure. Amongst recent authors Hedon (14) and Foa (15) subscribe to the theory of purely central origin of bradycardia, while Eyster and Hooker (16), as well as Tournade, Chabrol and Marchand (17), believe it to be based on a dual mechanism involving a central and a reflex stimulation of the vagus. Anrep and Starling state that "there is at present not sufficient experimental evidence in favour of any reflex mechanism being involved." Heymans, however, finds that the bradycardia and the vagus tone alike are purely reflex. It must be added that MacLeod (18) and Stewart and Pike (19) regard the vagus tone as being reflex, while Tiegerstedt (20) ascribes it to a central stimulation. In view of all these contradictory statements

with regard to Marey's Law our first problem was to pursue the enquiry by means of our new technique

The central mechanism

Our experiments show, contrary to Heymans' observations and in confirmation of the results of Anrep and Starling, that with the vagi and sympathetic nerves intact a rise of the blood-pressure in the head is followed by slowing of the heart rate. The central mechanism thus becomes obvious as a factor of the Marey's Law, since the conditions in the heart itself with regard to output, arterial pressure and temperature remain constant throughout the experiment. Moreover, the slowing of the heart rate on raising the cerebral blood-pressure and the acceleration when the pressure in the head is diminished does not depend on the rate at which the change in pressure is produced. A quick rise of the cerebral pressure has the same ultimate effect as a rise produced slowly. Once the heart rate has been changed, either by a rise or by a fall in the cerebral pressure, it continues to beat at its new rhythm so long as the cerebral pressure remains unaltered. The effect of a fall of the cerebral pressure cannot be explained on the basis of an inadequate blood supply of asphyxia since changes in pressure of not more than 15–20 mm. of mercury have often a considerable effect on the heart rate, while they have only a small effect on the blood flow through the head. In experiments in which the brain remains in a good condition the effect of changes in the cerebral pressure upon the heart rate can be observed at any stage of the experiment. The sensitivity of the brain to changes in blood-pressure varies from experiment to experiment. In most cases, however, we found that changes below a pressure of 80–100 mm. of Hg had little or no effect on the heart rate. Changes produced in the higher ranges of pressure progressively increase in their effect. For instance, in many experiments, a rise of the cerebral blood-pressure from 60 to 100 mm. retarded the heart rate by only 10 to 20 p.c. while a rise of pressure from 140 to 170 mm. caused nearly a complete arrest of the heart lasting for a considerable time. Fig 3 B and Exp 1 illustrate the effect of changes in the cerebral pressure upon the heart rate.

Exp 1 Output of the heart 468 c.c. per min. The systemic blood pressure is maintained between 90 and 105 mm. of mercury

Cerebral blood pressure	50	75	100	110	125	140	160	180	200	10
Heart rate per min.	204	190	174	162	126	102	66	30	0–18	228

The systemic blood pressure dropped below 60 mm. when the cerebral pressure reached 200 mm. of mercury

The reciprocal nature of the central regulation of the heart rate Cooper⁽²¹⁾ found that a temporary occlusion of both carotid arteries caused an acceleration of the heart beat. François-Frank ascribed this effect to a stimulation of the sympathetic fibres, Hunt⁽²²⁾ and later Sciliano⁽²³⁾ explained the acceleration by a diminution of the vagus tone, while Schiff and Navalichin⁽²⁴⁾ found the acceleration to persist even after injection of atropine or after section of both vagi. Hunt and Sciliano, as well as Kish and Sakai⁽²⁵⁾, ascribe the effect to a diminution of the vagus tone and a simultaneous increase of the sympathetic tone. The acceleration of the heart rate is generally considered to be due to asphyxia of the brain or to the fall of cerebral blood-pressure. The opposite effect of a rise in the arterial blood-pressure was ascribed by those authors who believed in the central origin of the effect either to the diminution of the vagus tone or to increase in the sympathetic tone.

In our experiments we found that the effect is chiefly due to a stimulation of the vagus centre but to a small extent also to a diminution of the tone of the accelerator centre. The part played by the latter can be noticed after section of both vagi or injection of atropine, when a rise in the cerebral pressure still causes a slight retardation of the heart beat (Exp 2)

Exp 2	Output of the heart 404 c c	Systemic blood pressure 100 mm					Both vagi cut	
Cerebral blood pressure	100	100	170	200	200	90	90	
Heart rate per min	192	192	183	176	180	196	196	

After extirpation of the stellate ganglia as well as section of both vagi the retardation disappears completely. Extirpation of the stellate ganglia alone does not change the effect to any appreciable extent. Since the changes in the cerebral circulation were never such as to cause any anæmia of the brain, it must be concluded that a rise in the blood pressure in the brain besides stimulating the vagus centre also inhibits the centre of the accelerator nerves, and conversely a fall in the cerebral pressure stimulates the sympathetic centre and inhibits the vagal centre. The central mechanism of regulation of the heart rate is therefore of a reciprocal nature.

A rise in the cerebral pressure produced by an injection of a small dose of adrenaline caused in our experiments an extreme slowing of the heart beat (Fig 2). The blood flow through the brain was in most cases affected only slightly or not at all, so that asphyxia could not occur. Similarly to the slowing of the heart produced by the mechanical rise in the cerebral blood-pressure, the slowing produced by adrenaline is mainly due to an increase of the vagus tone but also to a diminution of the sympathetic

tone After section of both sets of nerves adrenaline injected into the cerebral circulation has no effect upon the heart rate

The effect of adrenaline upon the central nervous system can be observed at any stage of an experiment, provided the brain is in good condition. The slowing of the heart is not completely abolished by artificially maintaining the cerebral pressure at a constant level. However, this does not imply a specific action of adrenaline upon the vagus centre since as shown by Anrep and Starling the pressure in the brachio-cephalic artery does not necessarily run parallel with the changes in pressure in the circle of Willis

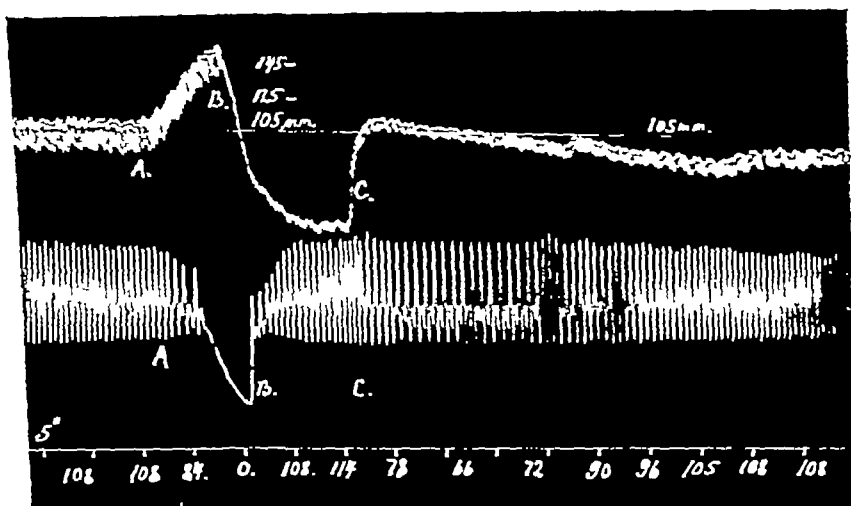


Fig 2. Output of the heart 560 c.c. blood flow through the head 180 c.c. per min. At A injection of 0.2 c.c. of 1:100,000 adrenaline into the cerebral circulation. At B the cerebral blood pressure is reduced artificially so as to bring about a return of the heart beat to its previous rate. At C the cerebral pressure is returned to normal the heart rate however remains slow for a long time and returns only gradually

The tone of the accelerator nerves The fact that the sympathetic fibres play a part in the central regulation of the heart rate makes it necessary to assume an existence of a definite tone of accelerator nerves. The presence of such a tone was obvious in most of our experiments and this could be shown in two ways, as follows

1. An extirpation of both stellate ganglia led in almost every experiment to a retardation of the heart rate after a preliminary quickening. This retardation was most marked when the removal of the sympathetic ganglia was performed after the section of both vagi

2 After section of both vagi the heart assumes a rate which is quicker than that generally observed in a denervated heart-lung preparation. If after section of the vagi the circulation in the head is stopped and the whole preparation thus transformed into a denervated one, the heart after a considerable acceleration assumes a rate which is far below the one before cessation of the cerebral circulation (Exp 3) The temperature of the blood in the heart and the mechanical conditions of the cardiac circulation remain in these experiments unchanged

It must be noted that the experiments with the removal of the stellate ganglia were performed under good circulatory conditions with a good oxygen supply to the brain In one of the experiments in which the sympathetic tone was very obvious the blood in the cerebral circulation was 98 p c saturated with oxygen and contained 47 vol p c of CO_2

Exp 3 Both vagi cut. Output 456 c c Systemic pressure 92 mm The heart rate keeps steady at 198 beats per min After arrest of the circulation in the brain the heart rate per min was (readings taken every 30 sec) 210, 222, 234, 240, 252, 252, interval 3 min., 220, 204, 204, 198, interval 3 min., 186, 180, 180, 174, interval 3 min., 152, 152, 152

The effect of anoxæmia upon the vagus centre Experiments upon the vagus centre under different tensions of oxygen and CO_2 will form the subject of a further communication In the present paper we shall describe only the effects of inadequate blood supply or insufficient oxygenation of the blood In our experiments anoxæmia was produced either by a temporary arrest of the cerebral circulation or by an insufficient oxygenation of the blood In the latter case the artificial respiration was changed from 60 p c oxygen to air containing in both cases about 4 p c of CO_2

The observations upon the effect of a sudden cerebral anæmia did not present any new points The first and immediate effect is always an acceleration of the heart which is best explained by the sudden fall of the cerebral blood-pressure After about 1 minute the vagus centre is re-excited, the heart slows considerably and if the anæmia is prolonged may stop, soon, however, the vagus centre becomes paralysed and the heart now under the influence of the accelerator nerve beats at a much faster rate than before the cerebral anæmia If the anæmia is produced after section of both vagi only an accelerator effect is observed This shows that in the first case both centres are being simultaneously stimulated by the anæmia, the effect of the inhibitory centre predominating over that of the accelerator Thus the two centres lose under these conditions their reciprocal relation

Readmission of blood quickly removes the effect of anæmia causing

an acceleration of the heart rate when the vagi are still active, and a retardation after their section or their paralysis. In both cases re-admission of blood removes the excess of tone of the centres. Exp 4 gives an example in which the blood on readmission was at a much higher pressure than before the anæmia. It can be seen that the excessive tone of the vagus centre was removed as soon as the period of anæmia was terminated: the heart accelerated its beat much beyond its rate before the period of anæmia. Soon, however, the vagus centre again entered into a hypertonic state, responding to the mechanical influence of the high pressure so that the heart rate came back again practically to the same extent as during the period of anæmia. This hypertonic state was now removed by dropping the cerebral pressure to its original level (Exp 4).

Exp 4. Output of the heart 768 c. c., systemic blood pressure 100 mm. Readings every 30 seconds.

Cerebral blood pressure	106	0	0	0	0	172	172	172	172	172	106	106	106
Heart rate per min.	136	198	152	88	60	192	195	136	98	98	136	142	142

We believe that experiments of this kind explain some of the contradictory statements made with regard to the central effect of blood-pressure. They show that the vagus centre can be in at least three different states: (1) when it is excited by high blood-pressure, the accelerator centre losing some of its tone and both centres causing a retardation of the heart, (2) when the vagus centre is excited by cerebral anæmia, the sympathetic centre being excited simultaneously, and (3) during the transition stage from stimulation by anæmia to the stimulation by high pressure, when the centre has lost its excessive tone created by the anæmia but is not yet responsive to the effect of high blood-pressure.

In those experiments in which anoxæmia of the brain was produced by insufficient oxygenation of the blood without changing the CO₂ tension in the respiratory air or the blood supply to the brain, it was found that these three different states of the vagus centre were more pronounced and succeeded each other in gradual stages.

The first effect of anoxæmia on the vagus centre is found in the alteration of its response to mechanical changes in the blood-pressure. Increases in the perfusion pressure are found to have a progressively smaller effect until, finally, they cease to have any effect at all. Often the heart is at this stage accelerated, and we are unable to state whether this acceleration is due to a diminution in the vagus tone or to increase in the sympathetic tone. On prolongation of the period of

anoxæmia the vagus centre is re-stimulated, the heart rate slows and now every rise in the blood-pressure causes an acceleration of the heart beat by removing the excessive inhibitory tone. Section of both vagi performed at this stage causes an extreme acceleration of the heart which is now subjected to the unrestrained influence of the excited accelerator centre. Increases in the cerebral pressure performed now retard again the heart beat by removing some of the excessive tone of the accelerators (Exp 5)

Exp 5 Output of the heart 540 c. c. Systemic pressure 92 mm.

1 Respiration with 60 p c. O ₂ and 4.2 p c. CO ₂		2 10 mins. after changing to air and 4.1 p c. CO ₂		3 15 mins. after		4 20 mins after		5 After section of both vagi	
Cerebral blood pressure	Heart rate	C B P	H R.	C B P	H R.	C B P	H R.	C B P	H R.
105	126	105	140	105	146	105	78	105	223
160	66	105	140	160	140	160	120	160	186
105	130	160	120	105	136	105	88	105	222
—	—	105	146	—	—	160	132	—	—

Group 1 represents the normal response of the vagus centres to an increase in the cerebral pressure

Group 2 shows the first effect of anoxæmia in that the effect of a rise of the cerebral blood pressure is diminished

In Group 3, which is recorded 5 minutes later, the effect of raising the blood pressure is absent.

Group 4 shows the slowing of the heart which is due to the stimulation of the vagus centre by anoxæmia, a rise in the cerebral blood pressure relieves the state of anoxæmia and instead of slowing the heart further now produces an acceleration.

Group 5 is recorded immediately after section of both vagi but with continuation of anoxæmia, a rise in the cerebral blood pressure removes the excessive tone of the sympathetic nerves and again reduces the heart rate

As stated above we have not studied the effect of oxygen lack and of CO₂ separately, and we are not in the position to state at present even the approximate oxygen saturation which is necessary for the maintenance of the vagus centre in a normal condition

After complete denervation of the heart cerebral anæmia has no effect on the heart rate

The evidence presented thus far leads us to the conclusion that an increased cerebral blood-pressure has under physiological conditions an antagonistic effect upon the vagus centre and the sympathetic cardiac centre, increasing the tone of the first and diminishing the tone of the second. A mild degree of cerebral anoxæmia gradually renders the vagus centre insensitive to changes in the arterial blood-pressure

Prolongation of the anoxæmia or greater degrees of anoxæmia stimulate both centres so that they tend now to antagonize each other

We do not find any explanation for the negative results of Heymans except only in the damage which was produced in his case by section of the spinal cord, and which abolished the sympathetic innervation of the heart. With an inadequate blood supply to the brain, the blood flow of which he could not determine, the vagus tone might have been due to an anoxæmia which prevented the rise in blood-pressure from causing a retardation of the heart beat. Heymans mentions that in the early period of some of his experiments he obtained an indication of the central effect, a fact which he considers to be of no importance since it disappeared very soon. In our experiments the rise in pressure in the brain produced this effect normally, so long as the experiment lasted.

The reflex mechanism

(a) *The effect of changes in the aortic pressure* The innervated heart-lung preparation allows us to change the blood-pressure in the aorta and in the heart without simultaneously affecting the pressure in the brain. Our experiments complete the observations of Anrep and Starling in showing that Marey's Law is based not only on the central mechanism but also on a reflex mechanism. In confirmation of the observations of Heymans we find that a rise in the aortic pressure without any corresponding change in the cerebral pressure, produces a slowing of the heart beat (Fig 3 A and C). This slowing of the heart is of a reflex origin and entirely disappears after section of the vagi. After destruction of the sympathetic nerve supply the reflex remains unchanged, but after injection of atropine we still find in several experiments a small diminution of the heart rate following an increase in the aortic pressure. These experiments suggest that the reflex mechanism of the regulation of the heart rate like the central mechanism is most probably based on a dual innervation, the vagus and the sympathetic nerves working in a reciprocal manner. Since the reflex disappears after section of the vagi the afferent path most probably runs in these nerves. We did not perform any experiments with the object of determining the seat of the peripheral receptor organs. The existence of separate central and reflex mechanisms, which both play a part in Marey's Law shows a striking similarity between the mode of innervation of the cardiac inhibitory centre and the vaso-motor centre, both centres being under central and reflex control.

The reason why the results of the experiments by Anrep and Starling

were negative with regard to the reflex mechanism is not quite clear. But it is certain that the central nervous system in our experiments was

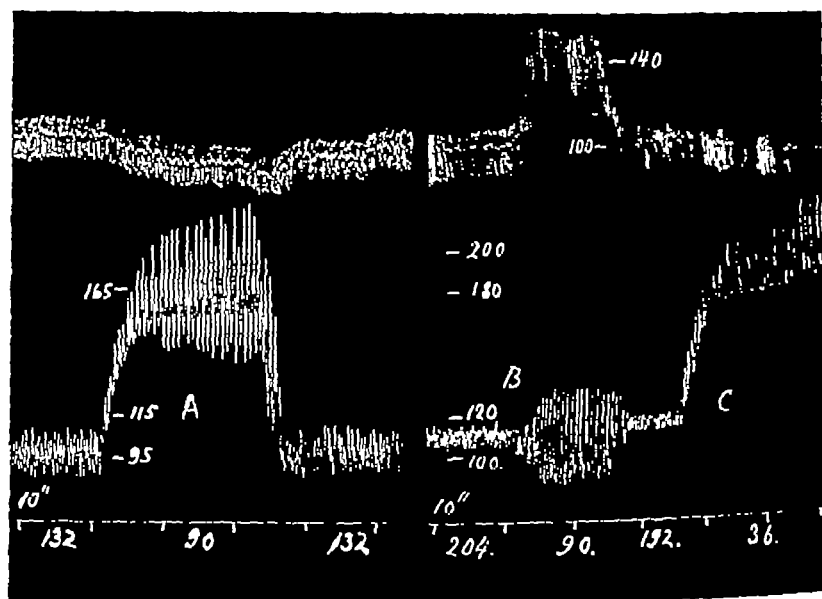


Fig 3 Showing the reflex retardation of the heart rate at A and C, and the central retardation at B. The two parts of the figures were taken from two different experiments.

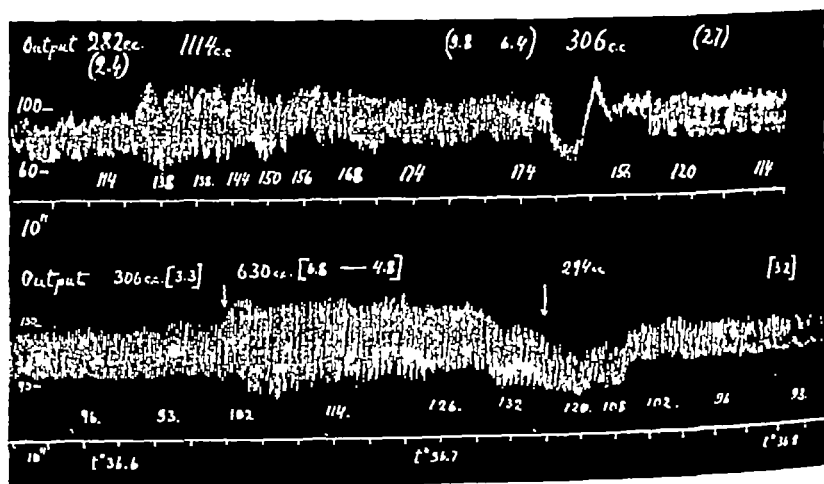
in a considerably better condition. Moreover, in our experiments not only was the brain but also the heart supplied with blood containing approximately a normal amount of CO_2 . There are also some definite conditions under which either the reflex or the central effect can be observed with greater facility. In cases when the vagus tone is reduced because of a low cardiac pressure we find it more difficult to produce a slowing of the heart by a rise of pressure in the brain (this was the case in most of the experiments by Heymans who worked on the spinal animal). If, on the other hand, the vagus tone is diminished on account of a low cerebral pressure it is more difficult to decrease the heart rate by the reflex effect of high aortic pressure.

(b) *The effect of changes in the output on the heart rate—the "Bainbridge reflex"*. It has been well established on the denervated heart-lung preparation that neither arterial pressure (and therefore the magnitude of the coronary flow), nor the changes in the output of the heart have any influence upon the heart rate. In the whole animal Bainbridge (20)

showed that the heart rate is affected by changes in its minute output, an increase in the output being followed by a considerable acceleration of the heart. This acceleration Bainbridge found to be based on a reflex which is initiated from the venous side of the heart due to increase in venous pressure. The afferent path was traced along the vagi, and the efferent mainly along the vagi, but also to some extent along the sympathetic nerves. The cardiac acceleration was thus the result of a reciprocal action of the two sets of nerves. Bainbridge obtained the increase in the output of the heart by means of injections of saline or defibrinated blood into the circulation of the whole animal. His experiments are open to criticism on account of the alteration of the composition of the blood, introducing changes with respect to its oxygen and CO_2 saturation, its H-ion concentration and its viscosity. Moreover, the changes in the output of the heart and in the venous pressure affected not only the venous side of the heart but also the circulation in the centres and in the lungs. Sassa and Miyasaki(27) confirmed the observation of Bainbridge, using rubber balloons to raise the pressure in the big veins and auricles. This eliminated the introduction of foreign fluids but introduced a number of other complicating factors. Since the Bainbridge reflex may be open to criticism on these grounds, we decided to perform a series of experiments upon this question. All our results showed definitely that the Bainbridge reflex does exist and plays a very important part in the adaptation of the heart to changes in the circulatory conditions.

Since we used the heart and lungs as a heart-lung preparation, it is obvious that any increase in the venous inflow into the heart within certain limits had no effect upon the arterial blood-pressure. Any rise in the arterial pressure accompanying very large increases in the inflow could be corrected practically instantaneously by adjusting the artificial resistance. The factor of temperature had also to be considered. According to Mansfeld(28) a sharp rise in the venous temperature of even less than one degree sets in operation an accelerating reflex. Kish and Sakai(29) deny that the acceleration is of a reflex origin and find no differences in the reaction of the heart to changes in temperature before and after denervation. Nevertheless, we thought it advisable to maintain the temperature of the circulatory fluid both in the heart and in the brain rigidly constant. With large flows this was not found to be difficult, and though in many experiments we observed changes of something less than $\pm 0.3^\circ \text{C}$ in others there was no change in temperature whatsoever. The Bainbridge reflex was observed in all these experiments. The

heart began to accelerate a few seconds after the output was increased, reached its maximum rate within 60 to 90 seconds and remained accelerated so long as the increased output was maintained. When the output was again reduced to its original volume the heart slowed down and reached its original rate within a couple of minutes. The acceleration was never such as to keep the output of blood per beat constant, but this was generally reduced by one-third or one-half of what it would have been without the operation of the Bainbridge reflex (Figs 4 and 5). After



As regards the location of the receptor part of the reflex our experiments are of a negative character. Bainbridge regarded the rise in the venous pressure as being the stimulus which sets the reflex in operation. He did not consider, however, several other factors which are involved in every case of increased output, namely, (1) increase in the diastolic volume of the heart, affecting the muscle and the visceral and parietal layers of the pericardium, (2) increase in the pulmonary pressure.

To describe briefly the results of our experiments, we can say that the reflex is unaltered after section of the pulmonary branches of the vagi and after removal of the parietal layers of the pericardium. On many occasions we observed a definite Bainbridge reflex with a minimum rise of the venous pressure, and in several cases the venous pressure did not rise at all or rose only for a short time. We therefore think that the question of the location of the receptor part of the reflex arc should be still left open and that it is premature to regard the venous pressure as being responsible for the reflex.

APPENDIX

The depressor and the pressor reflexes

Some vascular reactions were observed in the course of the preceding experiments, which though not subjected to special study speak in favour of the existence of a distinct depressor and pressor reflex as advanced by Pavlov⁽³⁰⁾ and by MacDowell⁽³¹⁾. Anrep and Starling showed that a sharp fall in the aortic pressure is accompanied by a rise in pressure in the perfused upper part of the body. This effect could be explained either by a diminution of the depressor tone or by a separate pressor reflex. In several of our experiments we obtained the same effect but could not decide with certainty between the two possible explanations. However, we have recorded up to the present three different experiments in which it was possible to determine the threshold blood-pressure in the aorta at which the depressor reflex is set into operation. In this way the depressor mechanism could be separated from the pressor reflex. Changes in the blood-pressure above 120 mm. caused in one of these experiments a definite depressor action, variation in pressure between 80 and 120 had no depressor effect. We can conclude, therefore, that the threshold aortic pressure of the depressor reflex was in this experiment about 120 mm. Hg. In the same experiment a fall of pressure below 80 mm. Hg was accompanied by an evident rise of the pressure in the head. Since the depressor mechanism was not involved

we believe that this increase in pressure speaks in favour of the existence of a separate pressor mechanism and thus supports the conclusion reached by Pavlov and MacDowell. Both reflexes disappeared after section of the vagi. Experiments of this kind do not bear out the suggestion that the natural thresholds of the reflexes are usually separated by a kind of gap in which neither one nor the other are stimulated.

CONCLUSIONS

1 The experiments described in this communication were carried out on the innervated heart-lung which is described in the text.

2 In confirmation of the experiments of Anrep and Starling and contrary to those of Heymans, the heart rate was found to be influenced directly by the blood-pressure in the brain. A rise in the cerebral pressure caused a slowing of the heart rate, an effect determined by a reciprocal action of the vagus and the sympathetic nerve¹.

3 Anoxæmia of the brain diminishes and finally reverses the central effect of blood-pressure upon the heart rate.

4 In confirmation of the experiments of Heymans and contrary to those of Anrep and Starling, the heart rate was found to be influenced also by reflexes arising from changes in the aortic blood-pressure. A rise in pressure caused a retardation of the heart.

5 The observation of Bainbridge that an increased output of the heart gives rise to a reflex acceleration of the heart beat finds confirmation in our experiments with the innervated heart-lung preparation.

We wish to express our thanks to Mr R. A. Nash for the very valuable assistance he rendered us during this work.

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¹ During the preparation of this paper a preliminary communication by Hering came to our notice in which he claims to have shown that the central effect of blood pressure upon the heart rate is based upon a reflex originating within the sinus caroticus, the afferent path being in the glossopharyngeal nerve. After destruction or denervation of the sinus changes in blood pressure had no effect upon the heart rate. In conjunction with R. A. Nash one of us (G. V. A.) found that after destruction of the sinus caroticus changes in the cerebral blood pressure continue, in the innervated heart-lung preparation to exercise the effect upon the heart rate as described in this paper.

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A METHOD OF MEASURING DIRECTLY THE TOTAL AND PARTIAL PRESSURES OF THE GASES IN BLOOD

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KROGH^(1, 8) described a bubble method for measuring the pressures of CO₂ and O₂ in blood coming from the blood vessels of an animal, and for measuring CO₂ in a small sample of blood. Although it might be possible to use Krogh's technique for the oxalated blood of man there are difficulties, viz (a) Krogh found that hirudin was necessary to prevent coagulation and that oxalate was unsatisfactory, (b) in our experience the blood above the bubble in the micro-analysis apparatus often becomes contaminated with the strong KOH or pyrogallol from below, so that it coagulates and the analysis is spoilt. Barcroft and Nagahashi⁽²⁾ have described another method for oxygen. There is some doubt how far this method can be applied to arterial blood if, according to Krogh, the total gas pressure is sometimes less than atmospheric pressure. Further, Barcroft and Nagahashi could not obtain satisfactory results for the CO₂, these were always too low.

We began by using Barcroft and Nagahashi's method, but the changes we have made in it have all been in the direction of Krogh's original method. The apparatus¹ consists of three parts which we have termed the syringe, the analyser and the absorber (Fig. 1).

The all-glass syringe, which is of the kind that is ordinarily used for washing out the urethra, has a ground glass plunger fitting closely into a glass barrel of capacity 15–20 c.c. The ends of the barrel and plunger are cone-shaped so that the dead space is axial and minimal. The barrel of the syringe is connected by a short glass tube of 2.5 mm. bore and 2 cm. length with a two-way tap. From this tap is a further length of 2.5 cm. of similar bore, having a ground glass taper end, which fits accurately into the cup of the analyser.

The analyser consists of a glass tube (length 8 cm. and bore 1.75 mm.) with a two-way tap. The proximal end is hollowed out into a ground

¹ The apparatus has been made for us by C. R. Müller, 6 Parton St. W.C. 1

glass cup for the nozzle of the syringe, to the distal end is fixed a piece of fine capillary tube of 0.25 mm diameter, 15 cm long and graduated in millimetres. Immediately before the beginning of the capillary tube

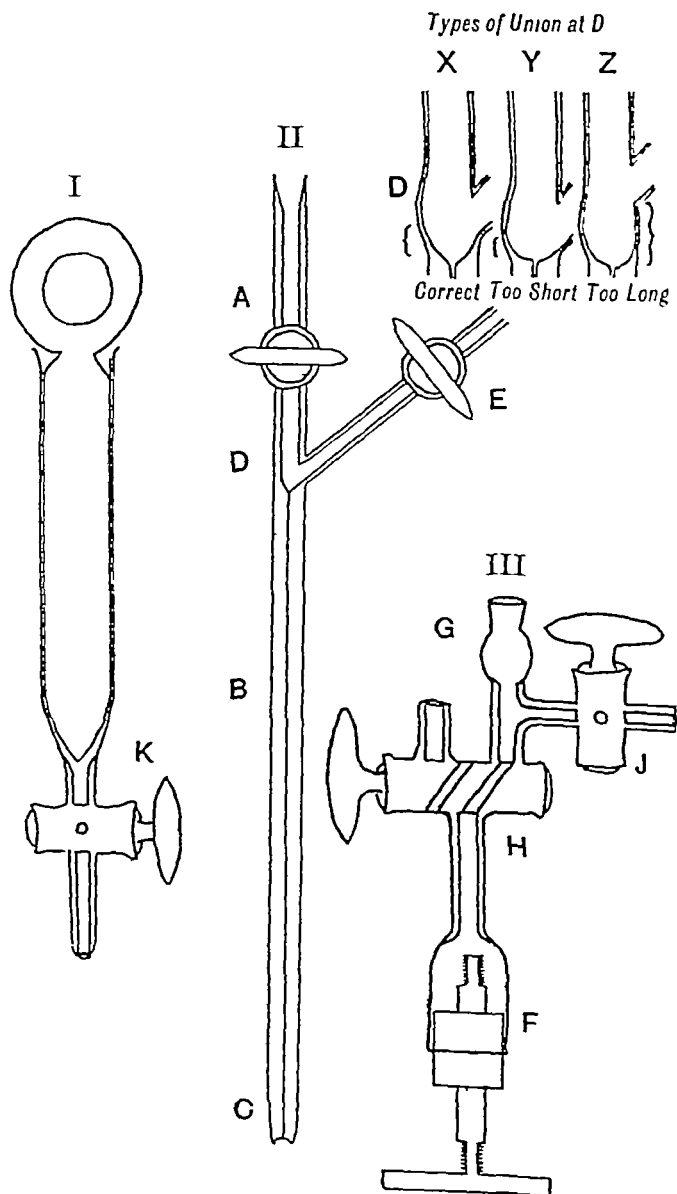


Fig. 1.

there is a slight enlargement, and at this point is fused a side tube of wide bore fitted with a two-way tap *E*. Great care is required to ensure a suitable union at the point *D* (see *X, Y, Z* in Fig 1). The distal end of the capillary tube has a fine ground taper which fits accurately into the proximal end *G* of the absorber, the actual end of the capillary being ground into a shallow saucer, as shown.

The absorber consists of a large three-way tap with three arms of wide bore tube, two on the proximal side and one on the distal. Of those on the proximal side one is a short straight tube serving as an overflow, whilst the other opens into a spherical chamber the mouth of which is ground for the reception of the taper end *C* of the capillary tube of the analyser. The distal tube is expanded to receive a 2 cm. rubber stopper fitted with a screw plunger. This latter consists of a fine threaded screw and gland set in a rubber stopper. This absorber resembles that devised by Barcroft and Nagahashi, but we have found it advantageous to add a short side tube with a two-way tap *J* between the bulb *G* and the tap *H*.

Use of the apparatus for partial pressures. The barrel of the syringe is coated with saliva as a lubricant preparatory to use¹. Mercury is drawn into the syringe which is then held upright with nozzle upwards and the plunger withdrawn until a little mercury escapes below. The plunger is then pushed vertically upwards, all air is displaced, and the tap turned off. The receptacle containing the blood is joined to the syringe by a short rubber connection filled with mercury, and the blood drawn into the syringe. In order to lubricate the tap it is gently loosened and a small portion of blood escapes round it. The syringe is now held vertically with nozzle downwards and the mercury allowed to run out until only a small portion remains to seal the exit tube. The fitting of a rubber cap allows the blood to be stored in ice till required.

The next step is the introduction of octyl alcohol and of a bubble of air into the syringe. A small portion of the mercury seal is replaced by octyl alcohol about 2 mm. in length. The mercury is now withdrawn until air occupies the distal 5 mm. of the tube, the syringe being dipped under mercury, the air and octyl alcohol are drawn into contact with the blood. All the mercury is expelled and the syringe is connected with the analyser, using a small portion of the blood to seal the joint. Rubber bands are then applied to *A* and *K* to hold the pieces of apparatus together.

¹ It is well to put some stiff grease round the top of the barrel of the syringe to prevent water from the bath being sucked in or blood being forced out.

With the apparatus in the horizontal position and taps *K*, *A* and *E* open, the blood is slowly driven into the analyser, care being taken that no bubbles of air remain adherent to the side. As the blood is driven along, it is used to lubricate the tap *A* in the manner described for tap *K*. When the bulb and the side tube are filled tap *E* is turned off and blood is driven into the capillary. To the distal end of the capillary is attached a length of clean dry pressure tubing having a tap *L* inserted 1 inch from the end of the analyser (Fig 2) and the tubing is filled with

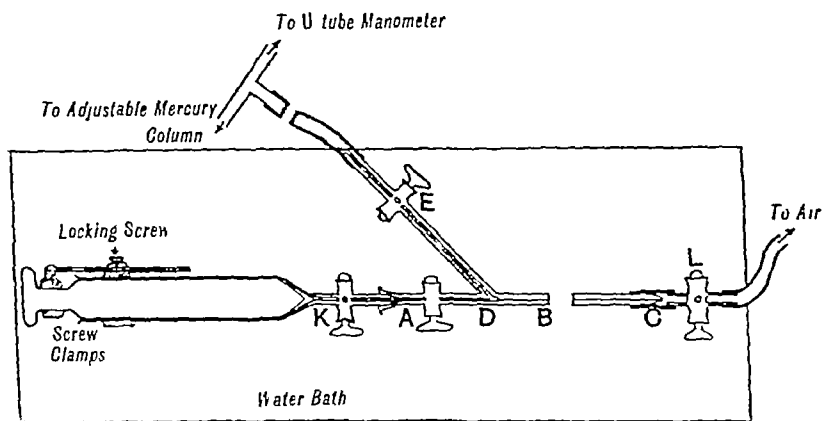


Fig 2 Diagram of Apparatus for Determination of Total Pressures.

blood up to the tap *L*. The distal end of the rubber tube is supported above the level of the water in the constant temperature bath. To the side tube *E* is attached pressure tubing which leads over the edge of the bath. This pressure tubing is of sufficient length to allow free manipulation of the apparatus and connects via a glass T-piece with (a) a mercury manometer, and (b) some air in an inverted burette fitted below with an adjustable mercury column, which we use for varying and measuring the total pressure, as described later.

The bubble is now ready for equilibration. The apparatus is immersed in a water bath electrically heated and kept at a fixed temperature (we used 37.5°C) by an automatic thermo-regulator. Taps *K*, *A* and *E* are opened, tap *L* closed, and the contents of the syringe being under a pressure closely approximating to atmospheric, the apparatus is rotated manually for 8 minutes. During this time the bubble is made to travel up and down the syringe by a slight tilting movement. The bubble is now to be driven into the analyser as a further precaution against the possibility of extraneous bubbles adhering to the side of the

tube containing our equilibrated gas, a small portion of the equilibrated bubble is detached and driven forward into the bulb, thus sweeping any such non-equilibrated air into the side tube *E* of the analyser. The main bubble is then driven forward, the apparatus being approximated to the vertical position to facilitate the passage of the bubble. When the bubble reaches the bulb *D*, tap *E* is turned off and tap *L* turned on, and by a slow, steady pressure the bubble is driven into the capillary until a suitable length is obtained. Tap *L* is now turned off and tap *E* turned on so that any small surplus may be driven up the side tube. Throughout these manipulations the apparatus is kept submerged in the water bath.

Micro-analysis The apparatus with taps *A* and *E* closed and *L* open is lifted out of the bath and the syringe and pressure tubing on *E* are disconnected. Tap *A* is opened and thereupon the analyser is immersed in a shallow water bath at room temperature, the whole manipulation being carried out with all possible speed. During the cooling of the analyser the tendency for the bubble to migrate up or down the capillary must be carefully watched and corrected. A small mercury seal is again drawn into the nozzle of the syringe, the cap is replaced and the blood can be stored in ice for a subsequent determination.

When the length of bubble has reached a constant value, this is noted—to avoid parallax the analyser rests on a plane mirror and the graduations are viewed through a reading lens. Taps *A* and *E* are now closed and the rubber tube with tap *L* is disconnected. The residual blood above and below the bubble has to be removed and replaced by normal saline, which has been shaken up with a few drops of octyl alcohol. For this the absorber is filled with mercury, and the plunger and cork fitted, care being taken to exclude all air bubbles. The mercury in the cup *G* is replaced by saline. The end *C* of the analyser is now inserted into cup *G*, the apparatus being tilted on one side and tap *J* opened as the analyser is forced home. Tap *J* is then closed and tap *A* opened. By manipulating the plunger the blood is drawn slowly from the capillary towards *G* until only a thin film of blood remains, care being taken that none of the bubble leaves the capillary. The bubble is now driven up the capillary by a column of saline until it reaches to within 1 cm. of the neck *D*. tap *H* is closed, and tap *E* opened. A wash bottle containing normal saline is connected with the side tube *E* and saline blown through to wash out excess of blood from *A*, *D* and *E*. When the fluid is clear the bubble is withdrawn into the centre of the capillary tube, taps *A* and *E* are closed, tap *J* opened and the analyser gently withdrawn. We have found it essential to leave about 1 cm. of blood in

the capillary tube adjacent to *D*, otherwise the washing causes some of the oxygen in the capillary tube to dissolve in the saline

To absorb CO_2 the saline in cup *G* is replaced by a solution of KOH (KOH solution 10 gm pure KOH in 55 c c water) all excess of saline in *C* is allowed to escape and the analyser reinserted into *G* with the precautions stated above Tap *J* is closed and taps *A* and *H* opened, and the bubble is slowly drawn down into cup *G* where it comes into contact with KOH on no account must the saline succeeding the bubble come into contact with the lower 2-3 cm of the capillary which is subsequently to be wetted by KOH from below The bubble is driven up and down 20 times and finally is left in the graduated part of the capillary tube and the whole apparatus placed horizontally on the mirror tap *H* having been closed, and taps *A* and *J* opened The length of bubble is read

To absorb the oxygen a similar procedure is adopted substituting for the KOH an alkaline solution of pyrogalllic acid This solution consists of 1 gm of pure pyrogalllic acid in 10 c c of the KOH solution To each of these solutions we add a few drops of octyl alcohol As the rate of oxygen absorption is slow the reading is not taken until after 80 descents of the bubble The further shrinkage in volume represents the amount of oxygen absorbed Since the best results are obtained with rapid working, the absorptions are not repeated

In order to test the accuracy of the method, samples of oxalated human venous blood were equilibrated in Barcroft tonometers containing mixtures of CO_2 and of O_2 according to our usual technique(3) The partial pressures of the gases in the blood were determined by the method described above and compared with the results from the Barcroft tonometers (Table I) During the analysis of the first bubble (B_1)

TABLE I Determinations of tensions of CO_2 and O_2 by bubble method compared with tensions in Barcroft tonometer (In mm. of mercury)

Date	Barcroft tonometer		Bubble method			
			1st bubble (B_1)		2nd bubble (B_2)	
	CO_2	O_2	CO_2	O_2	CO_2	O_2
22 xi 23	28.9	27.7	—	—	27.8	27.2
13 xii 23	44.8	54.4	42.7	50.3	—	—
20 xii 23	28.6	48.9	25.0	48.9	25.2	52.5
20 xii 23	31.0	53.1	28.5	—	28.0	56.5
2 i 24	44.0	26.1	47.5	26.8	45.5	28.7
2 i 24	48.2	36.3	49.3	33.6	45.0	33.0
1 vii 25	35.5	101.3	43.1	103.3	32.4	99.8

the syringe was kept surrounded by ice It was then equilibrated with a second bubble (B_2) on an average 2.6 hours later than the original

equilibration in the Barcroft tonometer, and again surrounded by ice. A third bubble was obtained and analysed after an average of 4.5 hours, from the same syringe of blood.

The degree of accuracy of the method in our hands is best indicated by Table I, which gives the results of seven consecutive determinations with oxygen pressures varying between about 30 and 100 mm. carried out during the last year and a half. We have only been able to work once a week and the intervals between the periods of work have been somewhat irregular. Greater accuracy might be attainable with more continuous work. However, apart from experiments, which must be neglected owing to some accidental source of error noted at the time, occasional inaccuracies for which there is no obvious explanation will always be met with in this kind of work, as for instance, in the CO_2 result of the first bubble obtained on 1 vii 25.

In order to get some insight into the effect of successive re-heatings of the blood on the pressures of CO_2 and O_2 in it, we have analysed the results of all our experiments successfully carried through at intervals during a total period of 38 months, but omitting those where the discrepancy was large. We have of course omitted an equal number at each end of the series, i.e. when the "bubble" tonometer value was much too high and when it was much too low. On comparing the arithmetic mean of the CO_2 results of B_1 against the mean of the Barcroft tonometer results, the former is 0.2 mm. lower, which indicates a very close agreement. The average CO_2 for B_2 is 1.8 mm. below the Barcroft tonometer value, and for B_3 2.9 mm. lower.

There would thus seem to be a slight fall in CO_2 with successive re-heatings and not a rise as would be expected from metabolic changes in the blood. Since the fall is continuous it may be explained by a gradual diffusion outwards of CO_2 through the saliva lubricating the piston. We hardly think it could have been due to the loss of CO_2 in successive bubbles, because even at the end of the last equilibration the volume of blood left in the syringe was always greater than the 2 c.c. allowed by Krogh. So far we have treated all the CO_2 results together, but in many of the experiments we added sodium fluoride to the blood to hinder metabolic changes, as suggested by Evans⁽⁴⁾. We mostly used 0.5 p.c., but in a few experiments we used 0.1 p.c., which is the proportion recommended by him. Our usual practice was to dissolve the fluoride and oxalate in the blood immediately after it was drawn, so that exactly the same blood was used in the Barcroft tonometer and our apparatus. In order to make the condition of these experiments resemble more

closely those met with when the gas pressures of body fluids are determined, we added an experiment (1 vii 25, Table I) in which defibrinated blood was equilibrated in the Barcroft tonometer, and this blood was transferred by means of an oxalated pipette (as in our usual practice of collecting arterial blood(5)) to the syringe which contained the requisite amount of fluoride, to make a 0.1 p.c. solution. Using Ellis's capillator method we found that no appreciable effect on the pH of blood was produced by adding 0.2 p.c. fluoride, though high concentrations did cause a fall of about 0.1 in the pH of a phosphate solution while oxalate caused no appreciable effect.

An additional factor must be considered when the oxygen pressures are compared. Since the top of the hæmoglobin absorption curve is very flat, a change of pressure between 80 and 100 mm. will not cause a big alteration in the volume of oxygen in blood, while above 100 mm. when the hæmoglobin is fully saturated the alteration in the volume of oxygen with pressure will be still less, since it depends solely on the solubility of oxygen in blood. Conversely, any alteration in the volume of oxygen due to metabolic changes will cause a much greater change in pressure above 80 mm. than below. For this reason we have considered our oxygen results in three categories, 0-80 mm., 80-101 mm. and 125-145 mm. (Table II). From 0-80 mm. in the case of B_1 , B_2 and B_3

TABLE II To show the effect of an increasing oxygen tension on the results obtained with the bubble method.

Oxygen pressure range	No. of determinations	Average oxygen tension in								
		Tono meter	B_1	Diff	Tono meter	B_2	Diff	Tono meter	B_2	Diff
0-80 mm.		48.8	51.6	+2.8	49.8	53.9	+4.1	60.3	63.6	-3.3
	Above tono meter value	—	5	—	—	10	—	—	9	—
	Below do	—	3	—	—	2	—	—	0	—
80-101 mm.		92.5	93.2	+0.7	92.8	93.5	+0.7	88.6	82.8	-5.8
	Above tono meter value	—	3	—	—	2	—	—	0	—
	Below do	—	1	—	—	1	—	—	3	—
125-145 mm. (fluoride blood)		132.9	137.5	+4.6	137.2	122.2	-15.0	146.1	116.8	-29.3
	Above tono meter value	—	2	—	—	0	—	—	0	—
	Below do	—	1	—	—	5	—	—	1	—

the average oxygen pressure is slightly above the Barcroft tonometer pressure and the difference is a little greater in the later bubbles, which suggests some diffusion of oxygen in from outside. (Certain experiments were spoilt by water from the bath obviously diffusing in along the piston, which greatly increases the oxygen pressure.) If there is any loss of

oxygen owing to metabolism, this is completely masked by the other error, and in any case the loss must be slight, since the effect of adding fluoride did not make any difference

We have four experiments, all quite satisfactory, carried out between 80 and 101 mm, two with fluoride and two without. The average difference for B_1 and B_2 is extraordinarily small, in each case 0.7 mm, which suggests that the two errors, if present, neutralise one another. With B_3 equilibrated after $5\frac{1}{2}$ hours, there is a loss of O_2 and a fall in pressure. This range is about that of the arterial pressure, and our technique is evidently satisfactory over this range.

Our oxygen results between 125 and 145 mm can be predicted from what has already been said. Even with fluoride blood there is a great falling off in pressure with B_2 and B_3 . The results for B_1 are not very concordant. We tested the effect of the absence and presence of fluoride at these pressures. The oxygen pressures in the Barcroft tonometer was in each case over 140 mm and the fall was certainly more rapid when no fluoride was added, and it was about the same with 0.2 p.c. fluoride as with 0.5 p.c.

The quantity of blood to be used in the syringe depends on the size of the bubble, and how often it has to be renewed owing to accidental loss during manipulation. We used 10 c.c. of blood for our analyses of three or four successive bubbles. We recommend in future the use of separate samples of 3 or 4 c.c. blood in equilibration with a single bubble each. An apparatus with an analyser of small bore has the advantage of requiring a smaller bubble, and the manipulation of the bubble is easier.

Finally, we recommend that the blood should contain 0.5 p.c. potassium oxalate and 0.1 p.c. sodium fluoride and be equilibrated for 8 minutes with 6 c.mm. air and about 3 c.mm. octyl alcohol. We found that it takes 5 minutes for the 10 c.c. blood in the syringe to be warmed to the bath temperature, after taking the syringe from the ice in which it has been stored.

We fully agree with Krogh that it is essential for the whole apparatus to be absolutely clean so that no minute drops of liquid can be seen on the walls of the capillary after cleaning, but we have not found the mixture of sulphuric acid and bichromate recommended by him at all effective. On the suggestion of Prof. C. S. Gibson we used a mixture of alcohol and nitric acid. We allowed the analyser to stand inverted in a beaker containing one part of alcohol, the end *C* of the analyser being connected to a water pump. Three parts of non-fuming concentrated

nitric acid were then cautiously added and on the first sign of reaction suction was commenced and the effervescing mixture drawn through the analyser. Owing to the explosive nature of the reaction the whole manipulation must be carried out in a fume cupboard. The process is repeated and the apparatus washed through until no signs of acid remain as shown by litmus. Lastly the analyser is dried by placing in a hot water oven and drawing air through it.

A little vaseline may be used to lubricate taps *E*, *L*, *J* and *H*, but for taps *A* and *K* which are in the direct course of the bubble, it is best to use the blood itself as a lubricant. Before cleaning by the above method care should be taken to remove all vaseline.

Method of determining the total pressure of gases dissolved in a fluid
Krogh, working on animals with his microtonometer(1), stated that the total pressure of gases (O_2 , N_2 , CO_2 aqueous vapour) in blood was sometimes as much as 120 mm Hg below that of the atmosphere. We have, therefore adapted our method to determine the total pressure of gases dissolved in the fluids under examination.

As Krogh pointed out(2), the equalisation of gaseous pressures between a bubble of gas and the gas dissolved in a fluid can only be complete when the total pressure of gas, i.e. the sum of the partial pressures, is the same in both cases. To take a concrete example, suppose we try to equilibrate a bubble which consists of N_2 of a partial pressure of 600 mm Hg, O_2 of 60 mm and CO_2 of 100 mm, i.e. a total pressure of 760 mm with a fluid in which the gaseous pressures are N_2 700 mm, O_2 60 mm and CO_2 100 mm, i.e. a total pressure of 860 mm or a positive pressure of 100 mm. At the beginning the O_2 and CO_2 are in equilibrium, but the partial pressure of N_2 in the fluid is greater than that of the bubble, so N_2 passes out of the fluid into the bubble and consequently its volume increases. This increase of volume diminishes the partial pressure of O_2 and CO_2 in it. This leads to the passage of O_2 and CO_2 from fluid to bubble and a further increase in volume. In this way a condition of "sliding equilibrium" is obtained in which the bubble steadily increases in volume and the pressure of the gases in the fluid is diminished. If the total pressure of gases in the fluid is less than that of the bubble, gas will be absorbed by the fluid and the bubble will steadily diminish in size. Equality of total pressure will result in no alteration in the size of the bubble.

The apparatus we have described above for measuring the partial pressures of gases in fluids, can be very readily applied to determine the total pressure of gas in a given fluid. The fluid to be examined is collected

in the syringe over mercury, as already described, the mercury is displaced and a small bubble of air, together with a little octyl alcohol, introduced. The syringe is then connected to the analyser and the fluid driven along until the capillary and side tube are filled. To the side tube *E* is connected a length of pressure-tubing leading *via* a T-piece to (a) an adjustable mercury column, (b) a U-tube mercury manometer. This enables the contents of the apparatus to be subjected to various total pressures recorded by the manometer (Fig. 2). The end of the analyser *C* is fitted with a short piece of pressure-tubing, a tap *L* and a length of tubing leading out of the equilibrating bath—this enables the bubble to be transferred to the capillary when the side tap *E* is closed. All the taps except *L* are left open and the mercury column adjusted to atmospheric pressure and so the contents of the apparatus, bubble and fluid, are under atmospheric pressure. The bubble is then equilibrated for 6 or 8 minutes and then driven up to *D*, tap *E* is closed and *L* opened, and the bubble is driven into the capillary and measured. It is then withdrawn into the syringe and equilibrated for a further 2 minutes and again measured. This process is repeated until a series of readings is obtained which gives a clear indication of the pressure conditions of the gases in the fluid—three readings are usually sufficient. If the bubble steadily increases in volume, the total pressure of gases in the fluid is greater than that in the bubble and the whole system must be subjected to an increased pressure, if the bubble shrinks a diminished pressure must be applied, if the volume remains constant the total pressure is atmospheric. Suppose the volume increases steadily, the bubble is withdrawn into the syringe, taps *E* and *L* are closed and the mercury column is adjusted to an increased pressure, *e.g.* 50 mm Hg. The pressure selected is decided by the degree of volume change, which gives some indication of the amount of pressure to be applied. Tap *E* is then opened and the bubble equilibrated for 2 minutes under this pressure. Then the process of measuring is repeated. A further increase in volume indicates an insufficient plus pressure and further measurements at a higher pressure are required until a series of readings is obtained in which the volume actually begins to diminish, *i.e.* the plus pressure applied is greater than necessary. In this way a "bracket" is obtained, one pressure being too high and one too low. The actual pressure can be determined by taking an intermediate value or by interpolation if the bracket is not too large. A similar procedure is adopted with a negative pressure if the original bubble shrinks.

When a positive or negative pressure is applied to the apparatus,

great care is required to maintain the piston of the syringe in a constant position as its movement alters the volume of the closed system and so of the total pressure applied. At first we relied on manual retention, but recently we have had light metal screw clamps (made in the Hospital Works Dept.) fitted to the piston and barrel of the syringe. The piston clamp carries a light metal rod which slides in a collar on the barrel clamp and can be locked in any position by a set-screw in the collar. Further, the application of a high negative pressure encourages leakage of water from the bath along the side of the piston into the syringe. To overcome this, when the apparatus is assembled, we apply a generous coating of thick vaseline to the piston at its point of entry into the barrel.

Theoretically we are continually altering the total pressure of the gases dissolved in the fluid during our efforts in finding it. Thus, suppose a fluid with a total gas pressure of 860 is equilibrated with a bubble 760 mm Hg, the total pressure of 860 mm is continually falling and the true total pressure is never obtained. Actually the alterations in total pressure in the fluid are very slight. For example, if there are 10 c.c. of blood in the syringe it will contain about 4 c.c. of gas whereas the volume of the bubble is 0.06 c.c. and the alterations in the size of the bubble are about 0.01 c.c. This small loss of gas by the blood can make no appreciable difference to the total pressure in the blood.

In order to check the accuracy of this method two series of experiments have been performed.

(1) To ascertain how closely the manometer readings indicated the actual pressure of the fluids in the syringe, we replaced the piston by a one-holed cork carrying a short glass-tube connected by pressure-tubing to a second manometer. This recorded the actual pressure in the syringe. The apparatus was filled with blood as usual and subjected to various pressures. A comparison of the applied pressures and those recorded in the syringe showed a very close agreement.

(2) Samples of blood were placed in a Barcroft tonometer and exposed to atmospheres of various pressures. The pressures in the tonometers were measured and compared with the results obtained by the above method. Table III shows that the pressures obtained by the bubble method are nearly always slightly below the correct values.

The method described gives reasonably accurate results in the determination of the total pressures of gases dissolved in a fluid. Krogh(7) has shown that small errors (7 mm Hg) in the determination of the total pressure do not affect the partial pressure determination of O_2 .

TABLE III Table of total pressure determinations.

Date	Observer	Pressure in Barcroft tonometer	Pressure determined by "bubble" method
25 vii 23	E C W	- 200 mm Hg	- 200 mm. Hg
23 i. 24	E C W	- 12	- 20
30 i. 24	E C W	- 110	- 120
12 iii 24	E C W	- 94	- 100
12 iii 24	W R S	- 57	- 58
	W R. S	- 60	- 65
19 iii 24	W R S	+ 11	\pm 0
		+ 58	+ 53
3 v 24	W R S	+ 8	\pm 0
4 vi 25	W R S	+ 45	+ 38
	W R S	+ 66	+ 24
10 vi 25	W R S	+ 52	+ 46

and CO_2 to any considerable extent. Larger errors (40 mm Hg) may give rise to an error of 4 p.c. in the O_2 determination. The errors of our method are of the first order and do not offer any serious bar to the subsequent determination of the partial pressures.

CONCLUSIONS

A "bubble" apparatus for determining the total pressure and the partial pressures of O_2 and CO_2 and N_2 in physiological fluids is described, with experiments showing the extent of its accuracy.

This work was begun during the tenure by one of us (E P P) of a Beit Research Fellowship. We should like to thank the Medical Research Council for financial assistance and the Royal Society for defraying the expenses of apparatus through a Government Grant.

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A METHOD FOR THE STUDY OF THE PERFUSED PANCREAS BY B. P. BABKIN AND E. H. STARLING

(From the Department of Physiology and Biochemistry
University College London)

THE activity of the pancreas is extremely susceptible to the conditions of its blood supply. It seemed to us of advantage to be able to have this factor under the control of the experimenter, and we have therefore devised methods for the perfusion of the pancreas by means of a heart-lung preparation. The two following methods have been employed

1 *Perfusion of the isolated pancreas and duodenum* The pancreas of a dog receives arterial blood from three sources (r Fig. 1) (a) a branch of

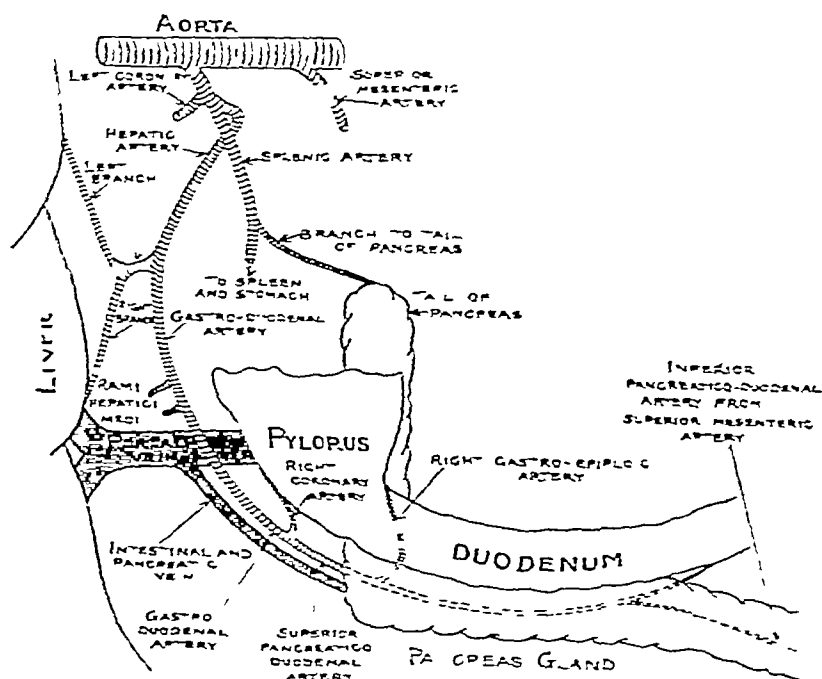


Fig. 1.

the splenic artery passes into the tail of the gland, (b) the body of the gland receives blood from the pancreatico-duodenal artery, which is

a branch of the hepatic branch of the *cœliac* axis, (c) the head of the gland is fed by a branch of the superior mesenteric artery

In a dog under morphua and chloralose the first and third arteries are ligatured a T-shaped cannula provided with a thermometer is inserted into the pancreatico-duodenal artery By this cannula part of the arterial blood from the brachio-cephalic artery of the heart lung preparation is allowed to flow into the arterial vessels of the pancreas The pancreatico-duodenal vein or the portal vein is cut A cannula is also introduced into the main pancreatic duct Into both ends of the duodenum cannulae are inserted, and the pancreas, together with the duodenum, is removed from the body, placed in a warm funnel, and the circulation is established The blood flowing out of the pancreas drops from the funnel and is restored at intervals to the venous reservoir By this method only the middle portion of the pancreas is kept alive It retains a normal pink colour to the end of the experiment, lasting three hours The preparation shows a very slow spontaneous secretion.

Exp 1 Temperature of arterial blood = 37° C. Blood pressure = 120 mm Hg Secretion noted every 2 minutes in the divisions of a graduated tube Time of collection of 10 c c of venous blood from the gland in seconds Intestinal juice in c c

Pancreatic juice	Venous outflow	
3	6	
4	6	
3	5 75	
2	6	5 c c. secretin added to blood in reservoir
3	5	
10	5	
13	4 5	
10	4 25	
9	5	
6	6	
4	7	5 3 c c intestinal juice secreted in 15 minutes
5	7	
4	7 5	
2	9	
		10 c c of 10 p c sodium bicarbonate added to blood
3	9	
2	8	
3	6 75	
2	6	
		5 c c. secretin added to blood
3	5 75	
13	5 25	
14	5	
11	5	
8	5	
7	5	
6	9	11 c c intestinal juice in 28 minutes
4	8	
4	8	

depending probably on the action of the chloralose. The addition of secretin to the blood in the venous reservoir gradually increases the rate of secretion as well as the blood flow from the gland. This is seen in the above experiment.

The pancreatic juice collected during the experiment (18 c.c.) contained traces of blood. It was fully active and digested fresh fibrin in $2\frac{1}{2}$ to 3 minutes, both in the absence and in the presence of intestinal juice. The perfused part of the duodenum is very active. It shows vigorous movements and secretes a viscid fluid which contains a great quantity of blood.

2 *Perfusion of the pancreas in situ*. In this case the perfusion of the pancreas with the arterial blood of the heart-lung preparation proceeds through the thoracic aorta (although it can be performed, as in the first case, through the pancreatico-duodenal artery). The abdominal aorta is ligatured below the coeliac artery. The following arteries are ligatured (see Fig. 1) left coronary, splenic, all the hepatic branches of the hepatic, right coronary and right gastro-epiploic, inferior pancreatico-duodenal artery. The venous blood from the gland is collected from the peripheral end of the portal vein. The cannula inserted into the portal vein is connected by means of rubber tubing with the venous reservoir of the heart-lung preparation. As an example we quote the following experiment.

Exp. 2 Both vagi cut in the neck of a dog the pancreas of which is perfused. Temperature of the arterial blood entering the aorta = $37.5-38^{\circ}\text{C}$. Blood pressure = 110-120 mm. Hg. The secretion of pancreatic juice before the administration of secretin was noted every 5 minutes, after that every 2 minutes. Time of collection of 10 c.c. of venous blood from the gland in seconds.

Pancreatic juice	6	6	6	8*	13	4	3	4	4	5 c.c. of secretin	2	17	44 etc.
10 c.c. blood out- flow in seconds	20	20	20	—	20	—	—	18	15	—	11	—	9 etc.

* Both splanchnics cut in the chest

SUMMARY

Methods are described for the perfusion of the isolated pancreas of a dog and the pancreas *in situ* with the blood of a heart-lung preparation.

THE NORMAL CO₂- AND O₂-TENSIONS IN THE TISSUES OF VARIOUS ANIMALS

By J ARGYLL CAMPBELL

(From the National Institute for Medical Research, Hampstead)

SOME results of observations on normal CO₂- and O₂-tensions in certain tissues of the rabbit and the cat were published about a year ago(1), the method used was injection of gas, usually nitrogen, into the abdominal cavity and under the skin, the tensions being estimated after equilibrium had been established with the O₂ and CO₂ in the surrounding tissue spaces and fluids. The tensions obtained were considered to be those existing immediately outside the cell wall. Owing to the injection of the gas causing a temporary hyperæmia, the true O₂- and CO₂-tensions were not obtained for a few days, in other words, until the hyperæmia had passed off. Results of further and more extensive observations from rabbits, cats, and from various other animals, using the same method, are recorded here. We shall deal first of all with homoiothermous animals.

Rabbits The average results for rabbits are arranged, in Table I, in two series, namely *A* and *B*. In series *A* are given the results of simultaneous estimations for the tensions under the skin and in the abdominal cavity. In series *B* the tensions under the skin only were studied. In both series the average CO₂-tension under the skin was 49 mm Hg. In series *A* the average CO₂-tension in the abdominal cavity was 47. It has recently been shown(2) that these figures for CO₂

TABLE I Normal tissue tensions (homoiothermous animals)

Animals	No of obs	Tensions under skin (mm Hg)		Tensions in abdominal cavity (mm Hg)	
		CO	O ₂	CO	O ₂
47 rabbits (<i>A</i>)	150	49	24	47	37
28 rabbits (<i>B</i>)	115	49	22	—	—
4 cats	23	42	23	41	29
2 cats	12	43	20	—	—
3 rats	3	52	22	52	33
3 rats	12	53	26	—	—
3 guinea-pigs	3	56	20	56	28
4 guinea pigs	9	48	19	—	—
2 hedgehogs	6	51	27	—	—
2 fowls	10	46	25	—	—
2 monkeys	14	49	40	49	40

tension in the tissues were very much higher than those for the arterial CO_2 -tension which was usually about 35 mm. Hg

The CO_2 -tension remained remarkably constant in any one animal from day to day, perhaps now rising, now falling a few millimeters, but remaining almost a straight line over weeks and months, as pointed out previously (1). There were, however, fairly marked differences between different animals as regards their tissue CO_2 -tensions, and I have, therefore, arranged the observations to show their percentage distribution within limits of 5 mm Hg

CO_2 tension under skin percentage distribution.

	Below	40 mm. Hg	40-45 mm	46-50 mm	51-55 mm.	56-60 mm
115 obs with rabbits		1 p c	12 p c	43 p c	35 p c	9 p c
150		2 p c	29 p c	44 p c	24 p c	1 p c

CO_2 tension in abdominal cavity percentage distribution.

150 obs with rabbits	2 p c	33 p c	46 p c	17 p c	2 p c
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It will be seen that in the greatest number of observations, i.e. about 45 p c, the CO_2 -tension was between 46-50 mm Hg. The wide differences between some of the animals were most likely due to differences in circulation, in condition of the tissues, and in sensitivity of the respiratory centre to CO_2 .

As with the CO_2 -tension, the O_2 -tension in any one rabbit remained remarkably constant over long periods of time. The average O_2 -tension under the skin was about 23 mm Hg (see Table I), whilst that in the abdominal cavity was about 13 mm higher, being 37 mm Hg. This was probably due to the better circulation in the abdominal cavity, yet the CO_2 -tension was only 2 mm Hg lower in the abdominal cavity than under the skin. There are other factors besides circulation which might affect the O_2 -tensions, such as local metabolism and reaction of the tissue, a more acid tissue would accelerate the liberation of O_2 from oxyhæmoglobin, a higher rate of metabolism would lower the O_2 -tension, etc.

The O_2 -tensions in different rabbits exhibited fairly wide variations, as shown below

O_2 tension under skin percentage distribution.

	Below	10 mm. Hg	11-15 mm.	16-20 mm	21-25 mm.	26-30 mm.	31-35 mm.	36-40 mm.
115 obs with rabbits		0.5 p c.	8 p c.	23 p c.	42 p c.	21 p c.	5 p c.	0.5 p c.
150		—	3 p c.	28 p c.	34 p c.	23 p c.	7 p c.	—

O_2 tension in abdominal cavity, percentage distribution.

	25-30 mm. Hg	31-35 mm.	36-40 mm.	41-45 mm.	46-50 mm.
150 obs with rabbits	3 p c	27 p c	41 p c.	23 p c.	6 p c

It will be observed that under the skin the O_2 -tension most frequently registered was between 21-25 mm Hg, whilst in the abdominal cavity the figure most frequently observed in different animals lay between 36-40 mm Hg

It is not likely that the very low figures under the skin, that is, below 15 mm Hg, were from normal animals. Probably there was some circulatory defect. In any case, such figures were rarely observed, in fact in only three animals. On rare occasions in rabbits the O_2 -tension under the skin approximated fairly closely to the O_2 -tension in the abdominal cavity.

Cats As with rabbits the average CO_2 -tension under the skin was very similar to that in the abdominal cavity, the figures being 42 and 41 mm Hg respectively. These figures were considerably lower than the average obtained for CO_2 -tensions in rabbits though no very decisive conclusion can be drawn, since only 35 observations were made with cats.

With regard to the O_2 -tensions under the skin, the cat and the rabbit resembled one another fairly closely both in respect to the average tensions and the limits observed. The average O_2 -tension in the abdominal cavity of the cat was distinctly higher than that under the skin, but not so high as in the case of the rabbit, the average figures being 29 and 37 mm Hg respectively.

Rats and guinea-pigs Fifteen observations were carried out with rats and 12 with guinea-pigs, the results showing quite a fair resemblance to those already given for rabbits. The averages for CO_2 -tensions varied between 48-56 mm Hg. The average O_2 -tensions for the skin of rats and guinea-pigs were 23 and 20 mm Hg respectively, the O_2 -tensions in the abdominal cavity were distinctly higher, being 33 mm Hg for rats and 28 mm Hg for guinea-pigs. In these animals only small quantities of gas were injected and the tensions estimated in 1 or 2 c.c. of gas.

Hedgehog and domestic fowl Some observations were carried out with these animals, the tensions under the skin being studied. The prickles of the hedgehog were cut short over a small area of the skin of its back. The tensions were much the same as those given already for the skin of rabbits, etc. The hedgehog was feeding well and not in hibernation. The average CO_2 -tension for the hedgehog was 51 mm Hg, its O_2 -tension 27 mm Hg. The fowl gave on an average 46 mm Hg for the CO_2 -tension and 25 mm Hg for the O_2 -tension.

Monkey and man Two monkeys (*Macacus rhesus*) were under observation for over six weeks. The average CO_2 -tension under the skin

was 49 mm Hg and that in the abdominal cavity was the same, the total number of observations being 14. It is interesting to note that the tissue CO_2 -tension in monkeys was similar to that in rabbits. The monkeys were fed mainly on a vegetable and fruit diet.

The average O_2 -tension in the abdominal cavity of the monkey was 40 mm Hg, that is, 3 mm higher than that for rabbits. The O_2 -tension under the skin of the monkey was also 40 mm Hg. It would seem, therefore, that there was a much better O_2 -supply for the skin of the monkey than for any of the other warm-blooded animals tested.

It was of interest to make similar determinations in man, and I have injected gas under the skin of the back of my own arm and that of another subject. The gas was rapidly dispersed owing to movement, consequently I studied chiefly the CO_2 -tensions a few hours after the injection. These are about the same as the alveolar—the arterial— CO_2 -tension, probably on account of the hyperæmia caused by the injection of the gas. In myself I followed also the O_2 -tension changes on one occasion for 48 hours and found that 24 hours after the injection of pure N_2 the O_2 -tension was 61 mm Hg, in the next 24 hours it had fallen to 43 mm. These were similar to the figures for O_2 -tension following the injection of N_2 in monkeys, so that perhaps the figures given above for the normal and constant O_2 -tensions of monkeys might do for man also. Further observations on man are contemplated. Results should easily be obtained if the part of the subject injected is kept still, for several days, to prevent rapid dispersion of the gas.

Poikilothermous animals. Some results were obtained from frogs, toads, snakes and a tortoise. Only a few c.c. of gas could be injected, the amount depending upon the size of the animal. Even if only a few c.c. were injected, sufficient remained up to six or seven days for analysis with fair accuracy. In even $\frac{1}{2}$ c.c., by mixture with nitrogen in the Haldane analyser, the CO_2 and O_2 contents were obtained within about 10 p.c. error, as a rule 1–2 c.c. were analysed. There was little difficulty in injecting the gas through a very fine needle, and care was taken to avoid stretching the skin tightly, in fact the gas spread out easily under the skin. The difficulty was to get the gas out again, all the animals were under observation for several weeks and no sample was withdrawn before five days after an injection.

Toads (Bufo vulg.) and frogs (Rana temp.) Four toads were used in the first experiment which was commenced in September, 1924. They were placed on moist leaves in a room at 17°C and kept there for three weeks. Then they were placed in a warmer room at 23°C for two weeks,

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It is not likely that the very low figures under the skin, that is, below 15 mm Hg, were from normal animals. Probably there was some circulatory defect. In any case, such figures were rarely observed, in fact in only three animals. On rare occasions in rabbits the O_2 -tension under the skin approximated fairly closely to the O_2 -tension in the abdominal cavity.

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and lastly they were exposed in a cold room to 3° C for three weeks. The results for CO₂- and O₂-tensions are given in Table II

TABLE II Toads Tensions under skin in mm. Hg

Animal	CO ₂			O ₂		
	3° C	17° C.	23° C	3° C	17° C	23° C
1	5	11	14	101	28	44
2	3	10	14	98	78	68
3	3	17	14	108	43	77
4	4	13	15	95	55	—
Average	4	12	14	100	49	63

There is a striking increase in CO₂-tension with rise of temperature and a marked decrease in O₂-tension, possibly due to a higher metabolism. Toads and frogs use the skin as a respiratory organ. Krogh(3) has shown that they often breathe more through their skin than by their lungs. The high figures obtained for O₂-tension are probably explained by skin respiration. It was of interest, therefore, to study also the tensions in the abdominal cavity of the frog or toad. Toads were found to be unsatisfactory because when handled they filled up their abdominal cavity with their lungs and it was not possible to inject gas into them in the usual way. Frogs (*Rana temporaria*), therefore, were employed and a few c.c. of N₂ were injected carefully. The average CO₂-tension in the abdominal cavity was 14 mm Hg at 17° C, that is, similar to the tension under its own skin and also to that given in Table II for the skin of toads. The average O₂-tension in the abdominal cavity of the frog was 28 mm Hg, whilst that under its skin was 48 mm Hg.

Recently Wastl and Seliškar(4) studied the CO₂-tension in the mixed blood of the bull frog (*Rana catesbeiana*) by an indirect method and calculated approximately that the arterial CO₂-tension was 22 mm Hg and the venous about 29 mm. These are higher than the figures I have obtained. My frogs were not anaesthetised, whereas their frogs were narcotised with 25 p.c. urethane. I found that urethane often caused a marked rise of CO₂-tension in rabbits(1). It is doubtful, therefore, whether the figures obtained by Wastl and Seliškar may be considered to hold for normal unanaesthetised frogs.

Observations were made also on three toads living in water at 15° C for three weeks. The average CO₂-tension under the skin was 7 mm Hg, whilst the O₂-tension was 57 mm Hg, that is, the CO₂-tension appeared to be lowered whilst living in water, whilst the O₂-tension was not altered materially.

The frogs and toads were not fed during the above experiments

Snakes (*Coluber flavescens*, *S æsculapī*) Two snakes were under observation (unfed) for six weeks at 15° C during November and December, 1924 Five estimations gave very constant results for each (see Table III) Only a few c c of N₂ were injected under the skin

TABLE III

Animal	Temp. of air ° C	No of obs.	Tensions under skin (mm Hg)	
			CO ₂	O ₂
Snake (1)	15	5	21	24
Snake (2)	15	5	20	32
Tortoise	15	2	23	20

Tortoise A tortoise gave results very similar to those for one of the snakes The tortoise was feeding all the time A few c c of N₂ were injected under the skin of a leg and left there for over a week

SUMMARY

In all the cold-blooded animals—toad, frog, snake, tortoise—the CO₂-tensions in the tissues (at room temperature) were much lower than those for warm-blooded animals—rabbit, cat, rat, guinea-pig, fowl, hedgehog, monkey—the averages for the former varying from 14 mm to 23 mm Hg, and for the latter from about 40 to 50 mm Hg The average O₂-tensions under the skin were very much the same (20–30 mm Hg) for the warm-blooded and the cold-blooded animals with the exception of the monkey, the frog and toad, in the monkey the O₂-tension under the skin was about 40 mm Hg and in the frog or toad about 50–60 mm Hg at room temperature

Observations on tensions in the abdominal cavity were few with cold-blooded animals With warm-blooded animals the CO₂-tensions were much the same as those under the skin, that is 40–50 mm Hg The O₂-tensions were about 30–40 mm Hg in the abdominal cavity, that is about 10 mm higher than under the skin, in the case of the monkey, the same figure was obtained for the skin and the abdominal cavity, namely 40 mm Hg

The limits I have obtained by the above direct method, for CO₂-tensions in the different warm-blooded animals are somewhat similar to those previously obtained (by other observers) for certain animals by less direct methods, *eg* analyses of venous blood and tissue fluids(1, 5, 6) It would appear that the O₂-tension in the tissues outside the capillary wall is not nil, as often stated, but usually about 20–

40 mm Hg, thus agreeing with the results obtained by Verzář(7) for some organs of anæsthetised cats No anæsthetics were employed in my observations

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AN ANALYSIS OF THE INITIAL HEAT PRODUCTION IN THE VOLUNTARY MUSCLE OF THE TORTOISE

By W HARTREE¹

(From the Physiological Laboratory Cambridge)

THE chief difficulty in the accurate analysis of the heat production of an isolated voluntary muscle is the extreme quickness with which events occur. It is possible, to some degree, to overcome this difficulty by improvements in the instruments used for recording the rise of temperature as discussed in a recent paper⁽¹⁾. A simpler method is, if possible, to utilise the muscles of an animal whose movements are naturally slower than those of the frog the chief animal hitherto employed in such investigations. The slowest moving of all animals readily available in this country is the tortoise and the following investigation has been concerned with the *biceps cruris* of that animal.

The speed of contraction in the muscles of frogs in good condition is always approximately the same. In tortoises however the speed varies largely from one individual to another. Possibly these differences may be connected with the hibernating habits of this animal possibly they may be due to the great variations in age of the individuals employed. In the slowest twitch hitherto encountered relaxation was not complete for 30 or more seconds. 10 seconds at least were required for the tension to fall to half its maximum value. This was at 13° C. at 0° C. the contraction would have occurred several times slower still. The animal from which this particular record was obtained was in excellent condition. Perhaps because of the very slowness of its individual muscle twitch it was able to maintain a contraction with great economy. The *biceps cruris* used throughout this investigation (see Bojanus⁽²⁾) in the ordinary size of tortoise obtainable in this country, is usually about 5 cm. long and averages in weight 400 mgrm. It is of two to three times the cross-sectional area of a single frog's sartorius and very uniform throughout its length. This uniformity of section is essential for experiments in which a control deflection must be made by electrical heating of the dead muscle, since otherwise the latter will not be uniformly

¹ Working for the Medical Research Council.

heated by the warming current. A single muscle only has been employed, and not a double one as in the case of the frog's sartorius preparation. The rise of temperature in a contraction of this muscle is not as great, under similar circumstances, as in the much more quickly responding frog's muscle. Ample sensitivity, however, is available in the myothermic instruments, and the rather smaller amount of heat is no very great disadvantage (see, however, below). Altogether the muscle is admirably suited for myothermic experiments. In most cases the quickness of response was much greater than in the one described above, but still much less than in that of a frog's muscle. In general we may say that what happens to a frog's muscle in 1 second occurs in a tortoise's muscle in a time of the order of 15 seconds. This enormously simplifies and improves the accuracy of the analysis.

With the apparatus already described (1) experiments were made to determine the course of the evolution of heat at three different temperatures, 5°, 10° and 15° C. It was found impossible to perform satisfactory experiments at 0° C. Eight reliable analyses were obtained at 5° C., with times of stimulation 0, 0, 0, 0, 0.1, 0.1, 0.2, 0.4 second respectively, 0 standing for a single shock. Seven reliable analyses were obtained at 10° C. with times of stimulation 0, 0, 0, 0, 0.05, 0.1 and 0.2 second, eight at 15° C. with times 0, 0, 0, 0, 0, 0.04, 0.05 and 0.10 second. The course of the evolution of heat is shown in Fig. 1 as the average of the several experiments performed at each temperature. For comparison the isometric tension curves at the three temperatures also are shown, averaged for the several experiments at each temperature by adding all the tensions (using percentage of maximum in each case) in the several curves at each quarter of a second and re-plotting. The experiments were all performed between March and May, this may be of importance since the condition of tortoises varies considerably during the year.

The heat rate curves of Fig. 1 are plotted to a scale in which the *total* initial heat production (*i.e.* the total production of heat during the contraction and relaxation as distinguished from the "recovery heat production" following relaxation) is denoted by unity. The initial heat rate is very high and lasts only for a very short time. The tension curves shown by broken lines are plotted to an arbitrary scale with unity as a maximum.

The most striking fact which emerges from these analyses is the way in which the heat is split up into two completely distinct phases. There is a large initial outburst of heat occurring in the first 0.2 second or less

after the stimulus, followed by what appears in the analysis to be a gap, during which practically no heat appears at all, and then followed by

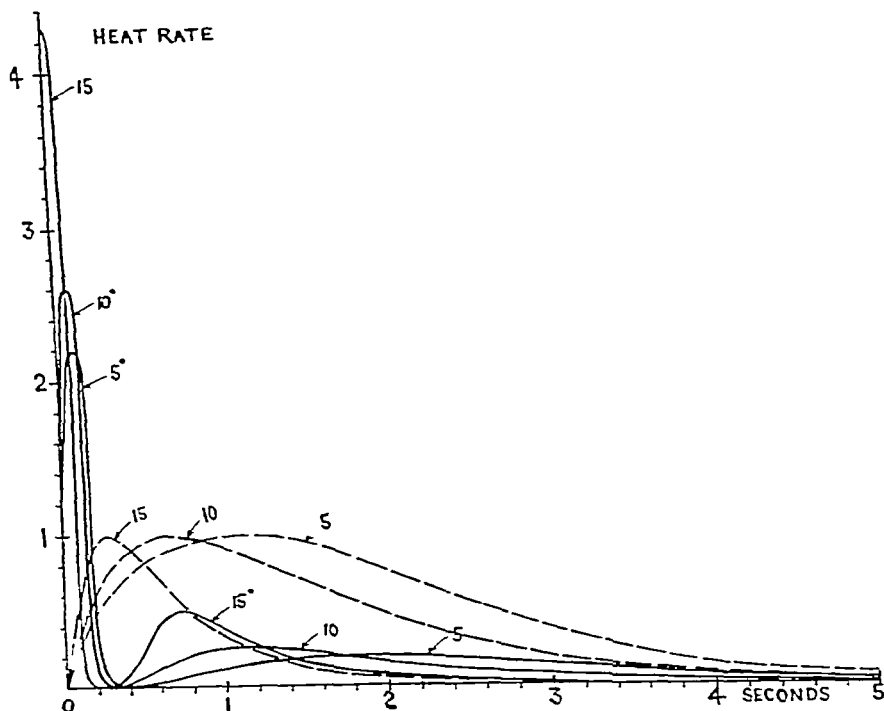


Fig. 1 Full line analysis of heat production in twitch, or very short tetanus, for comparison with broken line, mechanical response. Horizontally time in seconds. Vertically rate of heat production, or tension in isometric contraction. N.B. The unit of heat-rate is the total initial heat per sec. The unit of tension is arbitrary in each case.

a prolonged evolution of heat which continues as long as any tension is exerted by the muscle. In the analysis there is a sharp distinction between the two separate stages of the heat production, and it is possible to divide up the total heat in the initial stages of contraction quite definitely into its two separate parts. At 15°C the initial outburst of heat averages, in 14 separate sets of observations (including the eight most reliable experiments referred to above), for the case of a single shock or of a stimulus lasting not more than 0.2 second, about 49 p.c. of the total initial heat. The probable error of this mean value, calculated in the usual way, is about 2 p.c. At 10°C a similar series of ten sets of observations gave an initial outburst equal to 44 p.c. of the total initial heat, again with a probable error of the mean of about 2 p.c. At 5°C

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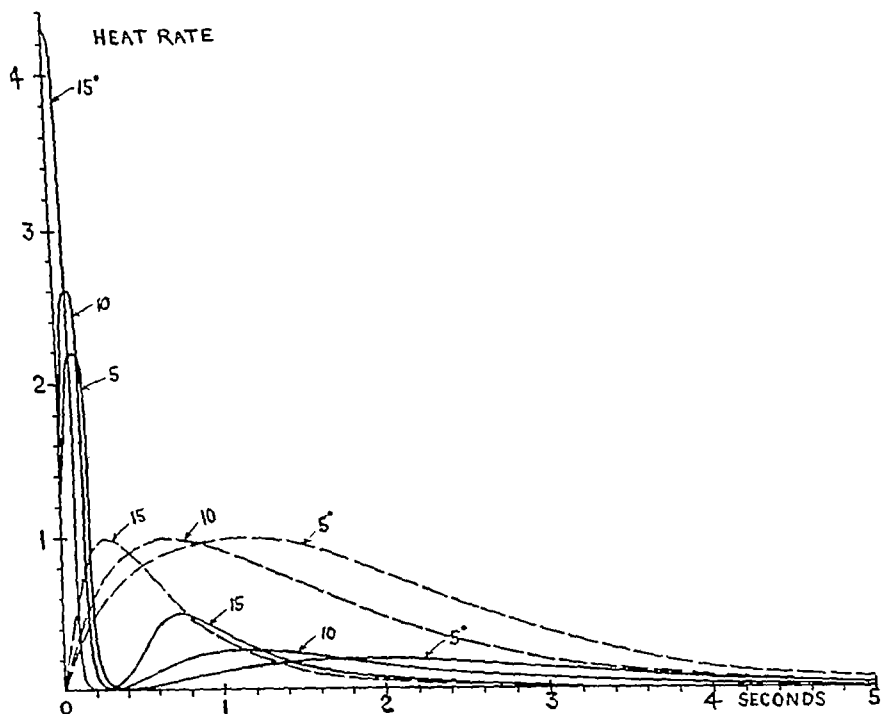


Fig 1 Full line analysis of heat production in twitch, or very short tetanus, for comparison with broken line, mechanical response. Horizontally time in seconds. Vertically rate of heat production, or tension in isometric contraction. NB The unit of heat-rate is the total initial heat per sec. The unit of tension is arbitrary in each case.

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a similar series of ten sets of observations, with stimuli of not more than 0.4 second duration, gave an average value for the initial outburst equal to 39 p.c. of the total initial heat, this time with a probable error of the mean of 2.5 p.c. It may be significant that the outburst of heat is rather a smaller fraction of the total initial heat at the lower temperature. This may, however, be due to chance, and with the number of experiments performed and their probable errors it is not possible to make any final statement on that point. It would seem certain, however, that in the contraction of a tortoise's muscle, in response to a single shock or to a very short tetanus, there is an initial outburst of heat completed in the first 0.1 or 0.2 second, which is about half the total initial heat production and occurs more rapidly at a higher temperature.

If the stimulus be of longer duration this initial outburst of heat is less clearly defined, and naturally forms a smaller fraction of the total initial heat. In many cases an analysis was carried out also of the heat production during a prolonged tetanic stimulus. Such analyses, however, led to no results of striking interest and need not be discussed here. It is sufficient to say that during the prolonged maintenance of tension there is a prolonged production of heat, and that during relaxation there is, as was to be expected, a continuous liberation of heat gradually dying away, as in the relaxation after a shorter stimulus.

After the initial outburst of heat is over the slower heat production, which previously was associated with relaxation (3), gradually works up to a maximum which occurs at each temperature at the moment when the tension is falling most rapidly. It then slowly decays as relaxation proceeds. In a general way, after relaxation has well commenced, the falling off of the tension runs parallel to the falling off of the rate of heat production.

It is tempting to discuss the chemical meaning of these two separate phases of the contraction. One would naturally tend to associate the sudden initial outburst of heat with the sudden formation of lactic acid from its precursor immediately following the application of a stimulus. At 15° C. at least a large part of this initial outburst occurs before the tension has risen appreciably. If we can imagine the reaction liberating lactic acid to be able to occur only during a short interval following a stimulus, for example, during a change of permeability associated with the presence of the action current, then we can understand the suddenness of this initial outburst of heat. Expressing the matter in terms of lactic acid after the acid has been produced it must do two things, firstly react with the substance of the muscle, for example with Garner's

liquid crystals(4) so as to cause the mechanical response, and then secondly be neutralised by the alkalis of the muscle substance. The process of reacting with the receptive material of the fibres or fibrils may be accompanied by a heat production which one would naturally place in the initial outburst. The neutralisation of the acid would inevitably be associated with a production of heat and this together with the dissipation of the mechanical energy in relaxation we appear to find in the second phase shown in Fig. 1.

In order to present the evidence more clearly and to give some idea of the magnitude of the errors attached to experiments and analyses such as these, an experiment is described in detail in the Appendix. In the individual experiments the same type of result is found as in the mean curve obtained by averaging a number of them. The errors attached to these observations are, as a matter of fact, rather larger than had been hoped. This is due in part to the fact that in the contractions provoked by short stimuli or by single shocks the heat production is less than in a frog's muscle, being only a few thousandths of 1°C , so that the instruments must be made more sensitive, less quickly reacting, and therefore more subject to error.

With the thermopile employed, which reacts very quickly to a rise of temperature and loses its heat also rather quickly, the total heat, if read simply from the maximum deflection of the galvanometer, may be appreciably in error when compared with the total heat as determined by a careful analysis of the photographic record. The heat production may be so prolonged, especially in contractions at the lower temperatures or in response to the longer stimuli, that a measurable fraction of the heat liberated in the earlier stages may have been lost before the maximum deflection is attained. The error so caused may, in a single twitch at 5°C , be as much as 20 p.c. of the whole heat. Usually it is much smaller than this in prolonged contractions; however, it may be even larger. In dealing therefore, with tissues producing heat only slowly one must beware of accepting blindly the maximum deflection of the galvanometer as a true index of the production of heat. It is easy to test an instrument for its efficacy in integrating the heat production over a long period by passing a constant calibrating current for different periods through a dead muscle on it and finding out the greatest period for which the deflection of the galvanometer is proportional to the time of passage of the heating current. By making a thermopile with the cold junctions far removed from the warm ones, and by means of thin wires and similar devices, it is easily possible to construct a thermopile which will go on integrating the heat production for a period of 10 or more seconds. The instrument used in this investigation did not do this, and it was always necessary when the production of heat was not very rapid, to take account of the possibility that some of the heat may be lost before the maximum deflection of the galvanometer is reached.

At the higher temperatures the initial outburst of heat may possibly occur in a time even shorter than the 0.1 or 0.2 sec. indicated above. The analysis would, in point of fact, be satisfied by a production of heat practically instantaneous and occurring at a moment shortly after the application of the stimulus.

APPENDIX

Exp of 21 iv 25 Single biceps cruris of tortoise in air, length 5.0 cm., weight 0.575 gm. Initial extension about 0.5 cm (very small initial tension) Galvanometer curves taken when contraction strictly isometric, tension curves taken separately Temp 5° C Stimulus 0.1 sec

Control curve, with time of heating 0.04 sec., mean of four records, very nearly the same Curve after stimulus, mean of two records, practically identical

Time (sec)	0	1	2	3	4	5	6	7	8	9	10	12	14	16	18
Control	0	4	32	93	176	273	374	477	568	649	721	826	903	947	972
Stimulus	0	1	4	19	43	73	108	142	178	212	244	301	351	396	435
Heat	12	22	01	0	0	01	02	—	05	—	03	04	03	03	02

Time (sec)	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.6	4.0	4.4	4.8	5.2	5.6	6.0
Control	987	997	1000	1000	998	996	993	973	960	946	932	919	905	
Stimulus	472	507	543	581	617	656	690	750	788	811	823	830	828	822
Heat	07	—	08	—	07	—	02	02	02	01	—	—	—	—

Control curves all reduced to maximum=1000 before mean taken (the unit being 0.1 mm on the photographic paper, corresponding to about 5×10^{-4} degree C in this experiment) Curves after stimulus both had a maximum=830 Result of analysis shown in the fourth line, the interval used at the start of the analysis being 0.1 sec., later 0.2 sec and at the end 0.4 sec. The tension in this case was very small after 6 sec., but just observable up to 8 sec The heat produced up to 4.4 sec. is 0.87 (the unit of heat being that which, if suddenly produced, gives a control maximum=1000, in this experiment it was 2.5×10^{-3} cal) The total initial heat is about 0.90 and it will be seen that there is 0.35 (i.e. 40 p.c of the initial heat) before 0.2 sec., and very little heat produced between 0.2 and 0.5 sec Thermopile used had 64 pairs of junctions constantan and iron, in one layer length (along muscle) 2.0 cm., and distance between "hot" and "cold" junctions 1.0 cm Resistance of thermopile 16.2 ohms, resistance of galvanometer 12.4 ohms. Galvanometer sensitivity 31 mm for 10^{-6} volt, with scale at 120 cm from mirror

SUMMARY

1 The skeletal muscles of the tortoise react very much more slowly than do those of the frog, a fact which simplifies and makes much more accurate an analysis of the heat production in the initial phases of contraction

2 There is an initial outburst of heat following rapidly on the stimulus and completed long before the tension has attained its maximum

3 Following this initial outburst there is an interval during which the tension is rising but no detectable heat production occurs

4 During and throughout relaxation there is a production of heat, reaching its maximum rate at the moment when the tension is disappearing most rapidly

5 Approximately half the initial heat is associated with each of these two phases

6 The chemical basis of these results is discussed

The expenses of this research have been borne in part by a grant from the Royal Society

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REACTION TO DRUGS OF STRIPS OF THE RABBIT'S GASTRIC MUSCULATURE

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THE movements and reaction to drugs of strips of the gastric musculature of the cat and dog have previously been described by us⁽¹⁾ The behaviour of the rabbit's stomach differs in many respects from that of the above animals and merits a separate description

Musculature of the rabbit's stomach The muscular coats of the stomach of the rabbit are arranged in three layers

(a) The external longitudinal fibres are thin and ill-defined, they are most marked along the curvatures and over the pyloric antrum

(b) The circular, or middle coat is the only complete layer The fibres of the fundus are arranged radially, arising from the cardia Over the lower two-thirds of the pyloric antrum the circular coat is exceptionally thick

(c) The greater number of the internal "oblique" fibres are collected in a distinct bundle on each side forming a sling The sling is less evident in the rabbit than in the cat and dog The fibres extend from the incisura angularis to the cardia, where they loop as a fairly broad band over the gastro-oesophageal junction Fibres pass out from the external edge of the sling throughout its length and run into the circular muscle, its internal edge remains well defined In the region of the lower body the majority of the fibres become continuous with the circular coat, but a small number appear to pass into the submucous tissue

Method The animals were killed by striking the occiput The stomach was removed and washed free of contained food in warm Tyrode's solution Strips consisting of mucous membrane, muscle and serous coat were taken in both longitudinal and circular direction in the following regions fundus, upper body, lower body and antrum and in the circular direction only from the cardiac and pyloric sphincters Records were obtained from the strips by suspending them in Tyrode's solution at 38° C, through which a constant stream of oxygen was led The apparatus has been previously described Pilocarpine nitrate and atropine sulphate were used in 0.1 p.c. solutions, a 1/1000 solution of adrenaline chloride

(Parke-Davis) was the source of adrenaline. In the majority of experiments 1 c c of the drug solution was added to the 250 c c of Tyrode's solution in the chamber, giving a dilution of 1 250,000. The addition of chlorotone in a concentration equal to that contained in the preparation of adrenaline was found to have no effect on the strips used.

RESULTS

The following description is based on the data obtained from experiments on 30 animals.

(1) *Spontaneous movements and reaction to pilocarpine and atropine*
Each region of the stomach possesses a characteristic type of movement. The records of the upper regions, fundus and upper body, are characterised by irregular contractions arising from an inconstant base line, while in the lower regions, lower body, antrum and pylorus, the base line is constant and contractions are regular in form and amplitude. Pilocarpine has a marked excitatory action upon all regions of the stomach with the exception of (a) subcardiac strips, (b) circular muscles of the anterior and posterior surfaces of the fundus, and (c) in the region of the lower body (Fig 5). In the fundus and upper body the drug produces a marked permanent shortening of the muscle, but has comparatively little effect upon rhythmic contractions (Fig 3). In the antrum and pyloric sphincter pilocarpine augments or initiates large contractions of the systolic and diastolic form, but has no effect upon the tone of the muscle fibres (Fig 6). Atropine antagonises the action of pilocarpine and has an inhibitory effect upon all regions. In the upper parts of the stomach atropine causes cessation of movement and

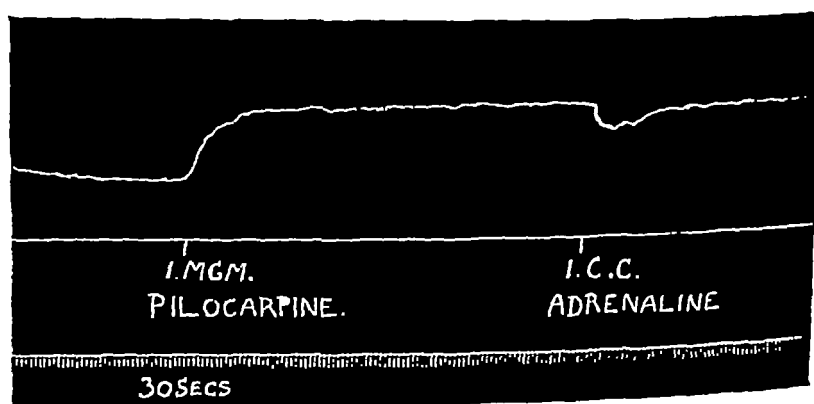


Fig 1 Augmentor effect of pilocarpine and inhibitor action of adrenaline on the cardiac

relaxation of the muscle, in the lower regions rhythmic movement only is affected. The cardiac and pyloric sphincters show no similarity in their reaction to pilocarpine and atropine.

(u) *Reaction to adrenaline* 1 *Cardia* The cardia of the rabbit is relaxed by adrenaline after addition of pilocarpine (Fig 1). If adrenaline be given in the first instance, the drug has no apparent effect upon the strip.

2 *Fundus* Strips from the fundus were taken in the following regions: (1) along the lesser curvature below the cardia—the subcardiac strip, (2) longitudinal muscle from the cardia to the apex of the fundus—the cardio-fundic strip, (3) circular muscle of the anterior and posterior surfaces, (4) longitudinal muscle of the anterior and posterior surfaces, (5) longitudinal muscle from the greater curvature.

The addition of adrenaline to these strips may be followed by either (a) a motor response, or (b) an inhibitory response.

(a) *Motor response* The addition of adrenaline to any region of the fundus may evoke a motor response. This effect is observed under all conditions in regions 1 and 3, and in strips 2, 4 and 5 when the muscle fibres show little tone. The motor effect is recorded as a sustained contraction, while rhythmic movements, if present, are unchanged or diminished. Should adrenaline be given subsequent to pilocarpine to strips 1 and 3, the motor effect of the adrenaline is superimposed upon that of the pilocarpine. It is significant that in these strips the addition of pilocarpine is not followed by a marked shortening of the muscle.

(b) *Inhibitory response* Adrenaline causes an inhibitory response of the muscle fibres of the fundus, (a) when the fibres are in good tone, and (b) after pilocarpine has been added to strips with little tone. Thus strips from regions 2, 4 and 5 which have previously given a motor reaction to adrenaline, are relaxed by the drug when the fibres have been shortened by pilocarpine (Fig 3). If strips from region (5) are in good tone, the inhibition is produced without preliminary addition of pilocarpine (Fig 4).

In regions 2 and 4 we have failed to obtain, without recourse to a stimulating drug, a preparation in good tone, although mixed reactions

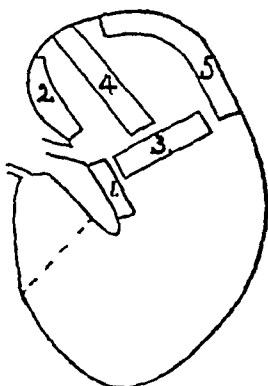


Fig 2 Regions of the fundus from which the preparations were made



Fig 3 Longitudinal strip from the greater curvature, motor response to adrenaline in atonic strip and inhibitory response with adrenaline after pilocarpine.

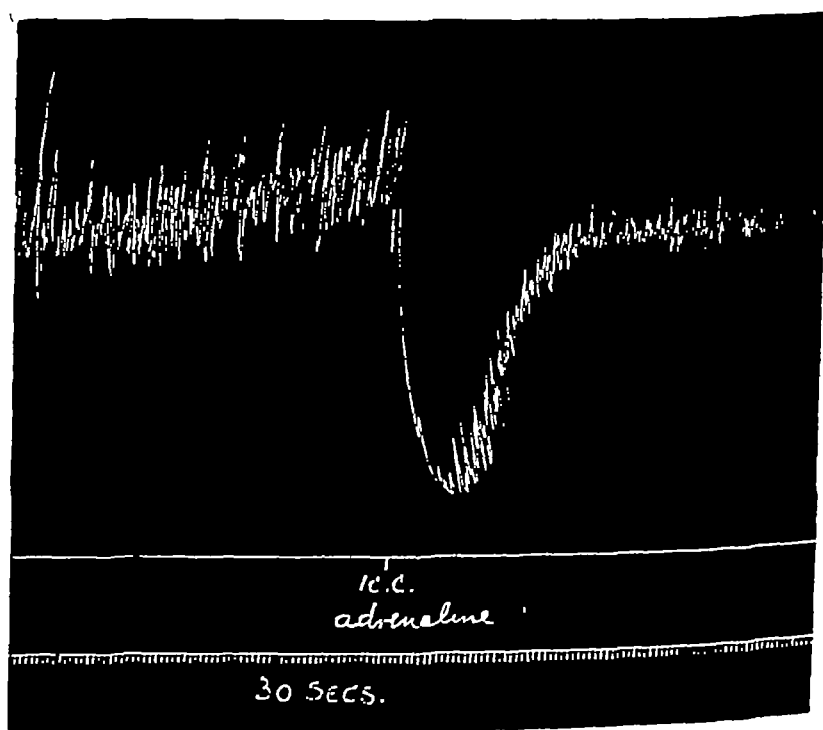


Fig 4. Longitudinal strip from the greater curvature, inhibitory response to adrenaline in preparation with good tone

were sometimes observed in which the motor response was preceded by an inhibition. The inhibition produced by adrenaline is recorded as an almost immediate and rapid relaxation of the muscle and cessation of rhythmic movement (Fig 3). The relaxation, although marked, is not of long duration in strips 4 and 5 and is often followed by an indefinite motor effect. In strip 2, the inhibition is of about 20 minutes' duration and no motor effect succeeds recovery.

3 *Upper body* The musculature of the upper body resembles closely that of the fundus in its reaction to adrenaline. The circular muscle gives a motor response under all conditions. An inhibitory response can be obtained in the longitudinal muscle, especially that of the greater curvature after the addition of pilocarpine.

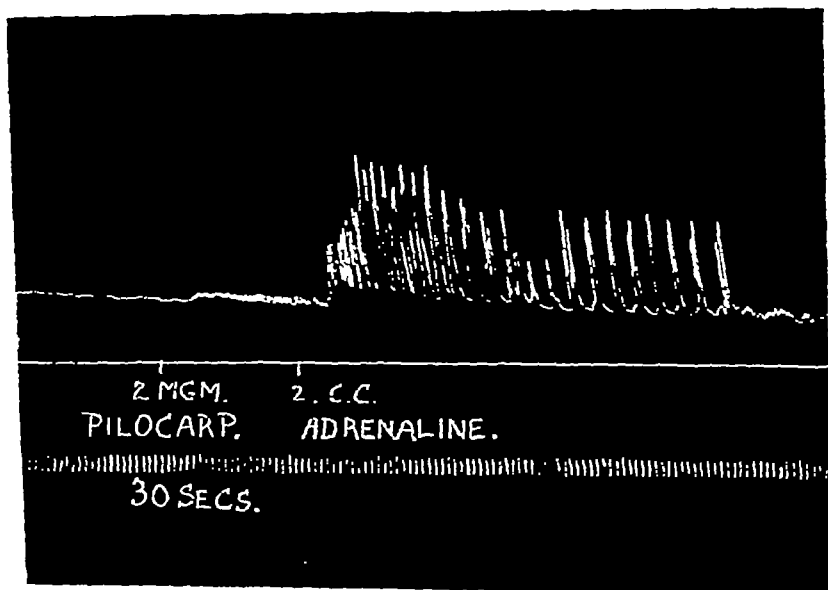


Fig 5 Lower part of body augmentation of contractions and slight shortening produced by adrenaline. Signal marker displaced 3 mm. to left.

4 *Lower body* Addition of adrenaline to the quiescent strip of the lower body causes a slight permanent shortening of the muscle and initiation of rhythmic movements, when given after pilocarpine it causes vigorous motor activity. There ensues a slight shortening of the muscle, contractions become much larger and assume the antral type of movement (Fig 5). It is important to note that pilocarpine has not a marked effect on this region of the stomach.

5 *Antrum and pyloric sphincter* Adrenaline has an inhibitory action upon these regions. Contractions are diminished considerably in amplitude and frequency. The permanent length of the muscle is unaltered.

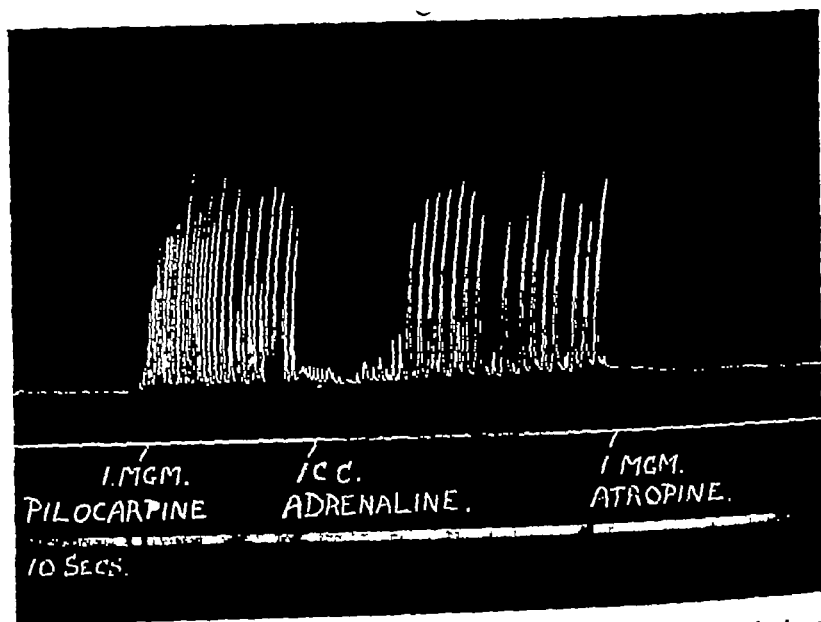


Fig 6 Inhibitory action of adrenaline on the antrum, the drugs affect rhythmic movements only and cause no change in the muscle length. Signal marker displaced 3 mm to right.

Occasionally in the antrum slight motor effects are observable, either preceding or following the predominant inhibition.

These reversal effects on smooth muscle are of interest as the same amount of adrenaline has been used to obtain the contraction and relaxation. The reversal effects recorded by previous investigators have been obtained in the main by using varying amounts of the drugs. The excitatory response of the gastric musculature of the rabbit to adrenaline has previously been described by Smith (2) and Kuroda (3) who, however, have not obtained reversed effects in the same preparation.

SUMMARY AND CONCLUSIONS

1 A sling formation of the internal layer of muscle fibres of the rabbit's stomach has been found similar to that in the stomach of the cat and dog.

2 Pilocarpine has an excitatory effect on all regions of the rabbit's stomach. In the upper regions the drug causes a permanent shortening of the muscle fibres while in the distal regions, rhythmic movements only are augmented.

3 Addition of adrenaline to strips from the fundus and body of the stomach may have an excitatory or inhibitory effect. The reaction depends on the condition of tonus, low tonus predisposing to an excitatory response, high tonus to an inhibitory.

The expenses of this investigation have been defrayed in part by a grant from the Government Grants Committee of the Royal Society.

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CARDIOVASCULAR REFLEXES BY I DE BURGH DALY
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DURING an investigation of the "Central and Reflex Regulation of the Circulation," Anrep and Starling⁽¹⁾ were unable to obtain evidence of a reflex mechanism being involved in the production of cardiac slowing in response to a rise in aortic pressure¹. They used the crossed circulation method described by Anrep⁽²⁾ in which the head derived its blood supply from a heart-lung preparation and the rest of the animal received blood from its own heart. It occurred to us that the negative results obtained might have been due to a rise in venous pressure occurring simultaneously with the rise in aortic pressure and so neutralising any tendency for the heart rate to become slower (Bainbridge⁽³⁾). The possibility that the low carbon dioxide content of the blood perfusing the brain contributed to their results was also considered, they did, however, use a carbon dioxide-air mixture for ventilation of the heart lung preparation in some of their experiments.

In order to study the problem further we devised a method in which the aortic pressure, the head arterial pressure and the head blood flow could be varied at will in the intact animal. Care was taken not to over ventilate the lungs.

Method. Dogs were used in all experiments and were fully anaesthetised with chloralose, 1 decigram per kilo body-weight being injected intravenously. Morphine was not administered. The blood of the animal was defibrinated and artificial respiration applied, a carbon dioxide mixture was not used. A screw clamp was placed on the thoracic aorta as low down in the chest as possible. A cannula was inserted into the central end of the subclavian artery and connected to a Pavlov and Stolnicov stromuhr^(4, 5), similar to the pattern recently used by Anrep. The brachio-cephalic artery was then tied close to its origin from the aorta and a cannula inserted pointing towards the brain, this was connected to the other end of the stromuhr. In two experiments the

¹ At a later date using the innervated heart-lung preparation with a CO mixture supplied to the lungs, Anrep obtained evidence of reflex slowing (Communicated to the Physiol Soc., Nov. 14th, 1925)

brachio-cephalic artery was divided between ligatures and cannulae inserted into the distal and proximal ends. These cannulae were joined to the output and input openings respectively of the stromuhr, in these experiments the left subclavian artery was ligatured. The blood flow through the head was controlled by a screw clamp on the rubber tube connecting the cannula in the central end of the subclavian artery (or of the brachio-cephalic) and the stromuhr. In some experiments the pressure in the right auricle was taken by means of a saline manometer, the upper end of which was in connection with a loose rubber membrane tambour. Mercury manometers joined by side tubes to the input (central to the screw clamp) and output connections of the stromuhr measured the aortic and head arterial pressures respectively. The stromuhr measured the amount of blood passing through the head, neck and right fore-leg.

The procedure was as follows. The blood flow through the head was measured and then the clamp on the aorta was partially screwed up. The clamp on the brachio-cephalic artery was then tightened to such an extent as to bring back the pressure of the head blood supply to its initial value, sometimes the aortic clamp and the brachio-cephalic clamp were screwed up simultaneously. The flow through the stromuhr was again recorded. Finally, both clamps were released and the flow through the stromuhr recorded.

In two experiments the temperature of the blood was taken in the right auricle, no alterations were observed as a result of adjusting the clamps as described. Reversing the direction of the blood flow through the stromuhr caused a slight kick on the blood-pressure records but did not influence the temperature in the right auricle or the heart rate.

Results Extracts from the protocols of three experiments are given in the table. It will be seen that a rise in aortic pressure with the head arterial pressure kept constant or even slightly diminished produced a slowing of the heart rate (Exps 1, 3). A rise in aortic pressure with the head pressure constant caused a diminished blood flow through the head, and if after raising the aortic pressure the brachio-cephalic clamp was adjusted so that the flow through the head was unaltered, the cerebral pressure was found to be increased (Exps 1, 2).

We obtained therefore, in response to a rise in aortic pressure, a reflex slowing of the heart rate and a reflex vaso-constriction of part or of the whole of the vascular bed supplying the head, neck and right fore-leg. After section of the vagi, the reflex slowing of the heart was abolished but the reflex vaso-constriction persisted (Fig 3).

The blood flow and the arterial pressure of the perfused head in the experiment published by Anrep and Starling were 335 c c per minute and 95 mm Hg respectively. In our own experiments 14 observations gave an average arterial blood-pressure of 91 mm Hg (limits 55-130 mm Hg) and an average blood flow of 75 c c per minute (limits 50-110 c c per minute), that is, a flow approximately one-fourth of that obtained by Anrep and Starling.

TABLE

Exp	Pressure in right auricle mm saline	M B P		Flow through head in c c per min	Heart rate per min	Temp °C. in right auricle	Time
1	10	126	126	50	180	—	—
			Aorta compressed				0
	16	154	160	—	108	—	15'
			Brachio cephalic artery compressed				1' 0
	12	128	180	—	102	—	1' 25'
2	—	126	170	35	126	—	3' 0'
	20	84	84	58	117	35	—
			Aorta and brachio cephalic artery compressed				—
	110	108	190	60	96	35	—
			Both released				—
3 a	20	70	70	—	126	35	—
	—	78	80	110	144	—	—
			Aorta compressed				0
	—	140	150	330	114-120	—	10'-50'
			Brachio cephalic artery compressed				1' 0
3 b	—	76	186	100	124	—	1' 10"-1' 45
			Both released				1' 55'
	—	70	70	110	140	—	—
	—	72	76	60	162	—	—
			Aorta and brachio cephalic artery compressed				—
3 b	—	64	190	45	120	—	—
			Both released				—
	—	60	60	54	153	—	—

Typical tracings (Figs 1, 2) show that the slowing of the heart rate in response to a rise in aortic pressure takes place almost immediately. If the aortic pressure was kept up for any length of time there was generally a progressive acceleration of the heart rate after the initial slowing, this acceleration was not sufficiently marked during the period over which our observations extended for the heart rate to return to the same value obtaining before the aortic pressure was raised.

In one experiment we used the same technique but in addition the lower half of the animal was removed by a section through the lowest thoracic vertebra, the aorta being connected to an artificial resistance and the inferior vena cava to a large rubber reservoir in the manner described by one of us (6) for making the closed circuit heart-lung preparation. On raising the artificial resistance and adjusting the clamp on

the brachio-cephalic artery so as to maintain the cerebral arterial pressure constant, we obtained a marked slowing of the heart rate—114 to 54 beats per minute

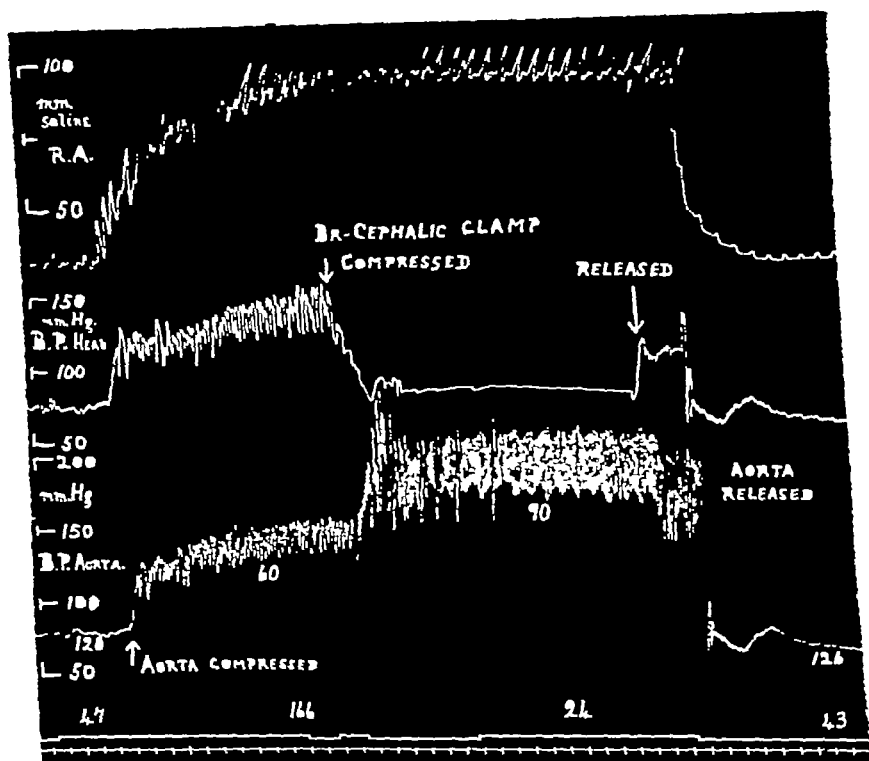


Fig 1 Partial compression of aorta followed by partial compression of brachio cephalic artery The figures below the aorta blood pressure tracing denote the heart rate per minute. Time tracing = 5 secs. *R A* = pressure in right auricle The figures immediately above the signal marker represent the blood flow through the head in c.c per minute.

Discussion

Our experiments demonstrate that a rise in aortic pressure causes reflex slowing of the heart when the pressure of the blood supplying the head, neck and right fore-leg remains constant. In those experiments in which partial compression of the aorta produced a rise of pressure in the right auricle, there was still cardiac slowing present, so it would appear that the reflex initiated by aortic pressure increase causing slowing of the heart is stronger than the reflex initiated by an increase in venous

pressure which causes acceleration of the heart rate. The reflex slowing is dependent upon the integrity of the vagi.

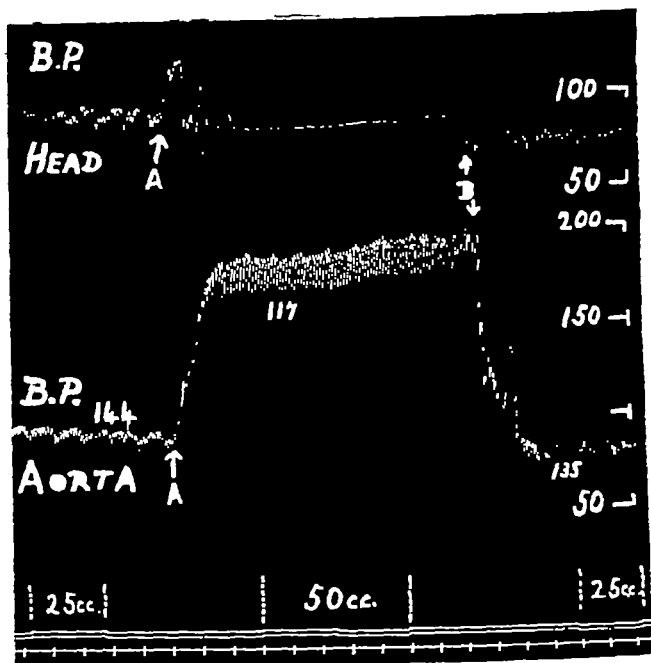


Fig 2 Simultaneous compression of aorta and brachiocephalic artery at A. At B both released. In this Fig and in Fig 3 the figures above the time marker denote the blood flow through the head during the interval between the dotted lines. Time tracing=5 secs

With regard to the accompanying vaso-constriction of part or of the whole of the vascular bed of the head, neck and right fore leg, we have not sufficient experimental evidence to account for its production. It occurs after vagal section when the stellate ganglia are intact, and in one experiment was accompanied by an acceleration of the heart rate. With the exception of the experiment in which we made the closed circuit heart-lung preparation, the suprarenal glands were intact, the possibility that adrenaline complicated our results was therefore not absolutely ruled out.

It is to be noted that the experiments which we have described do not decide as to the site of the receptors engaged in the reflex changes in cardiac rate. They may be in the aorta, in the heart, or even in the lungs.

CONCLUSIONS

1 A method by which the blood-pressure and blood flow to the head, neck and right fore-leg can be varied independently of the aortic pressure in the intact animal, is described

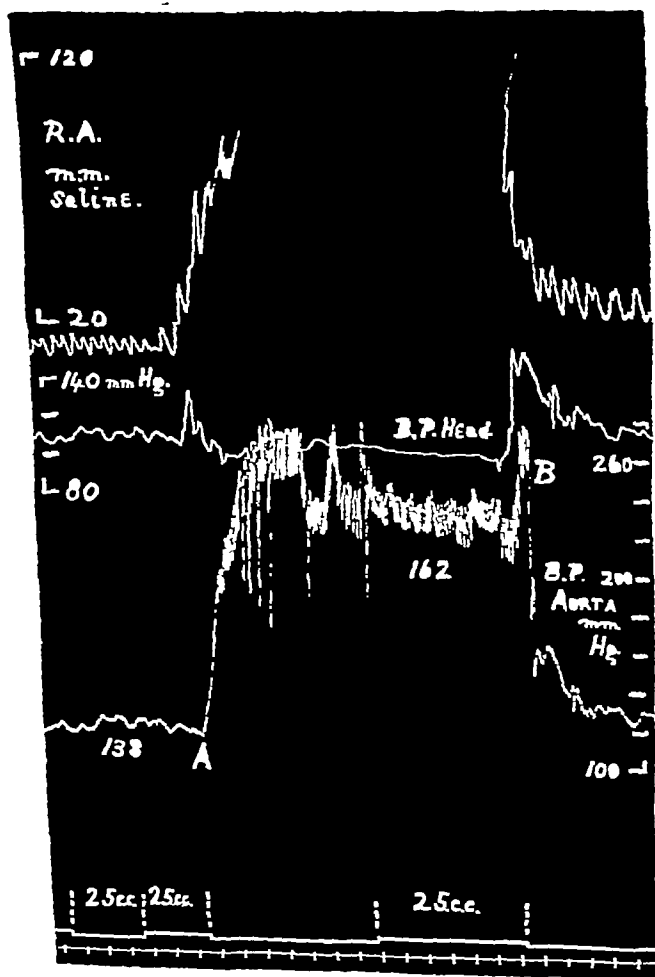


Fig 3 Same experiment as Fig 1 Vagi cut. At A compression of aorta and brachio cephalic artery At B, both released.

2 A rise in aortic pressure causes reflex slowing of the heart when the cerebral pressure is kept constant

The expenses of this work were defrayed in part by a grant from the Government Grants Committee of the Royal Society to one of us (I de B D)

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STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE

The effect of alteration of initial length on the tension produced on contraction By R J BROCKLEHURST

(From the Physiological Laboratory, St Bartholomew's Medical College, London)

THE energy set free by the isometric contraction of skeletal (1, 2, 3, 4, 5) or cardiac (6) muscle, depends upon the initial resting length of the muscle, the graph relating tension-increment on contraction to initial length shows that the energy set free first increases with increasing initial extension, and then declines with further extension (2, 4) The purpose of the present experiments was to find whether plain muscle exhibits similar properties

A difficulty is met with owing to the fact that plain muscle cannot be said to have any definite "unloaded length," but exhibits very great variations in length on the application of minimal loads, this was circumvented by starting with the muscle always definitely extended so as to produce a very slight initial tension when at its shortest length Fluctuations in tension due to variations of tonus were usually slight

Method The lower part of the cat's ileum was used throughout as a source of plain muscle (Crane and Henderson (7) found that the ileum in the guinea-pig is much more sensitive to a stretching stimulus than is the upper part of the small intestine) The cats were killed by bleeding immediately after the induction of complete ether anaesthesia A portion of the ileum, measuring between 2.5 and 4.0 cm in length, was removed immediately after death, washed thoroughly and placed with a minimum of manipulation in the recording apparatus, this consisted of a vessel of oxygenated Tyrode's fluid (without glucose) at 38° C, in which the intestine was suspended between a fixed isometric lever above (recording frontally on a slowly moving drum) and the hooked end of a glass tube below The glass tube was led out of the bath and rigidly fixed into a Palmer stand, by means of a worm gear this could be raised or lowered, thus shortening or lengthening the gut

After a preliminary rest of 10 minutes in the Tyrode bath, the lower attachment of the gut was adjusted so that the gut itself became just taut without producing any appreciable tension in the isometric lever,

at this point the effective "relaxed" length of the intestine was measured (to within 0.5 mm). The muscle was then stimulated to give a maximal contraction by the addition of a solution of "Ergamine Acid Phosphate" (B W), to give a concentration of one part of histamine in 1,000,000.

The use of a drug such as histamine has certain advantages over electrical stimuli when dealing with plain muscle. It is more readily applicable and more certain in its results, a maximally effective dose of histamine always giving a satisfactory and uniform contraction.

The response of the gut to the addition of histamine was recorded on the drum as an immediate powerful contraction (after a short latent period), followed by a series of strong rhythmic contractions, usually at the rate of about seven or eight per minute, these rhythmic contractions were never as powerful as the first contraction, and they gradually decreased in force, the tension in the muscle once more approaching zero at the end of a minute or two. After the greatest contraction had been recorded, the bath was emptied and the histamine removed by three washings with fresh Tyrode at the required temperature.

The gut was then lengthened by means of the worm gear and the whole process was repeated at different initial lengths, first lengthening the gut through a definite range, and then allowing it to shorten by similar stages back to its original length. At the end of the experiment

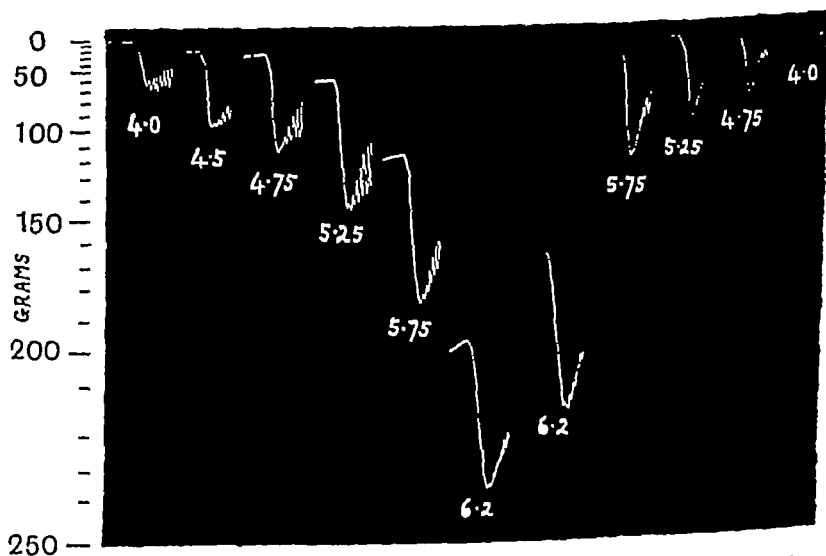


Fig 1 The figures indicate the lengths in cm at which the muscle was stimulated
Contraction downwards

the recording apparatus was calibrated by adding 10-gm weights to the isometric lever from the same point from which the gut had been suspended

Fig 1 shows portions of a typical record The length of the contracted intestine was not more than 0.25 mm shorter than when relaxed

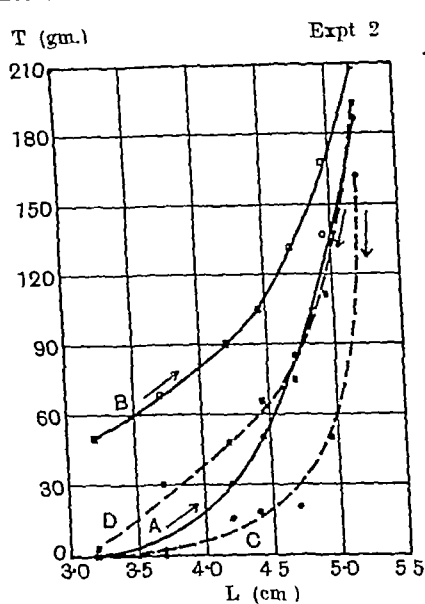


Fig 2

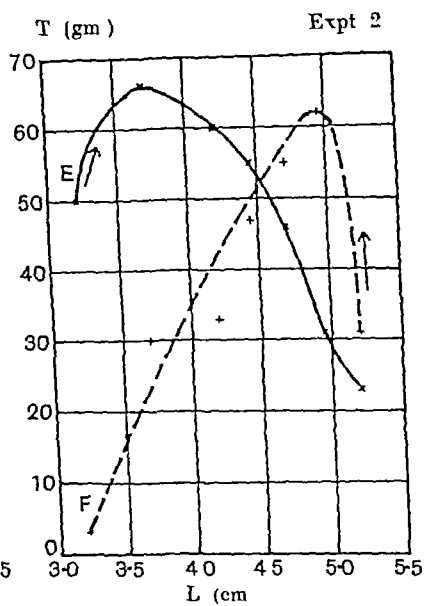


Fig 3

Fig 2 A = tensions when uncontracted, B = total tensions on contraction, when lengthening the gut, C = resting tensions, D = tensions on contraction, when allowing gut to shorten. Ordinates, tension in gm, abscissae, length in cm.

Fig 3 Drawn from data in Fig 2 $E = B$ minus A , $F = D$ minus C . Ordinates, tension-increment in gm, abscissae, length in cm.

Fig 2 represents graphically the results of a typical experiment of the series. The continuous curves show the effect of lengthening the gut by short stages, the broken curves represent the reverse procedure viz allowing it to shorten by stages to its original length. All the curves are of similar shape, differing only in degree. A and B are very similar to the corresponding curves obtainable with skeletal muscle, the writer has not seen any "shortening" curves (corresponding to C and D) in the case of skeletal muscle. Similar curves were obtained from all the experiments performed.

An obvious difference is seen between the tensions of the "lengthening" and the "shortening" curves, in every case the tensions on the

latter are lower than on the former at similar lengths. The shapes of *A* and *B* are similar, also those of *C* and *D*. The "contracted" curves *B* and *D* are clearly dependent for their shapes on those of *A* and *C* respectively.

There are a number of factors which, singly or collectively, may account for the difference in appearance between *A* and *C*.

(1) The intestine can, for the purposes of this investigation, be considered as a mixture of contractile and non-contractile tissues, the latter including the serous coat, the mucous membrane and a certain amount of connective-tissue in the submucosa and in the muscle layers. The sharper rise of *A* as compared with *C* is undoubtedly due partly to the presence of these non-contractile elements of low elasticity. On relaxing the gut, the stretched connective-tissue exhibits little or no retraction except at the beginning, and this would account, in part at any rate, for the sharp fall of *C*. The differences in level between *B* and *A*, and between *D* and *C*, are of course due entirely to the tension exerted by the musculature on contraction.

To avoid this difficulty if possible, a few experiments were performed with the retractor penis (bull and dog), which is as free from non contractile tissue as plain muscle from any other source. Unfortunately this muscle does not respond readily or in a regular way to histamine, relaxation being sometimes produced.

(2) *Viscosity*. On lengthening the muscle, there is a sudden increase in tension at the moment of lengthening, followed by a gradual decrease to a stable tension. It is obvious that only by stretching the muscle infinitely slowly can this factor be eliminated. On relaxation, the viscosity factor does not apparently alter the tensions appreciably, as the gut was allowed to shorten, it always assumed immediately a tension which did not vary thereafter. It would appear that whereas skeletal muscle can be compared to a thin rubber tube filled with a viscous mixture⁽⁹⁾, plain muscle in this respect would more closely resemble a similar system with very little or no elastic component. It is not necessary to enter here into the possible explanations of "viscosity" in plain muscle, whether it is due to a molecular rearrangement within the fibres, an adjustment of liquid crystals disordered by the stretching⁽¹⁰⁾, or to a movement of the fibres one over the other⁽¹¹⁾.

(3) *Damage*. In one or two instances a small degree of tearing at the ends of the gut was noticed at the end of the experiment, due to a partial rupture of the muscle coats by the thread used for ligaturing. This was obviated by fitting small flanged glass tubes into the ends of the gut and tying the gut over them. These tubes also served to keep

the ends of the gut open, thus allowing the free access of saline to its lumen, this enabled the gut to rid itself of metabolites, secretions of mucus, etc

(4) *Any other irreversible effects produced by extension* Under these experimental conditions there appeared to be no permanent stretching of the muscle, in one experiment (No 3 in the table) the gut was subjected to a second lengthening during which the tensions obtained were exactly similar to those during the first process

The muscular contraction itself is best studied by means of tension-increment—length diagrams

In Fig 3, *E* represents the mechanical energy liberated (as measured by the tension-increment) with increasing initial length, and *F* the same with decreasing initial length, *E* bears a close resemblance to the corresponding curve for skeletal muscle shown by Evans and Hill(2) There is clearly a certain optimum length for maximum tension development, and this optimum is different in the two cases (*a*) of muscle which is being lengthened and (*b*) of the same muscle being shortened A possible explanation of this difference is afforded by the behaviour of the non-contractile elements of the gut wall On lengthening the gut, *E*, the lack of plasticity of these tissues shows itself as a rapidly increasing rise in the resting tensions especially noticeable when it is considerably stretched, the effect of this is a falling off of the tension-increment The optimum length in *F* occurs very soon after the shortening process begins, and is always greater for a given piece of gut than the optimum in *E*, in this case the tension-increment is not interfered with by a stretching of the non-plastic tissues which are here allowed to relax

It may be noted in passing that the muscle of a small piece of intestine is capable of developing considerable tensions, in experiment 4, a piece of gut, 4.3 cm long, exerted a maximum tension-increment of 168 gm

Working with the gastrocnemius, Beck(4) found that the maximum tension developed on contraction occurred when the muscle was stretched to just beyond its greatest physiological length A V Hill(11) has emphasised the unsuitability of the gastrocnemius for experiments of this kind owing to the presence of tendon, etc, and to the obliquity of some of the fibres, he uses, instead, the sartorius and other skeletal muscles with parallel fibres The maximum physiological length of a given piece of plain muscle cannot easily be determined with any accuracy owing to the very indefinite range of movement of the 'insertion' with respect to the 'origin', e.g. the circular and longitudinal muscle coats of the hollow viscera There is no doubt that in the experiment

summarised graphically above, and in many of the others, the muscle was stretched beyond its physiological limit, and the tensions obtained from it when being gradually shortened may have been disturbed thereby. As mentioned above, however, a second lengthening process in one experiment indicated no permanent stretching effect. A few experiments were carried out in which the gut was not stretched by more than 25 p c of its initial length, curves drawn from these were found to be similar to those already described, with the exception that in some cases the maximum tension-increment was not reached.

The following table gives a summary of a number of experiments performed under similar conditions.

Expt	Original length cm	Optimum length increase	
		Up p c	Down p c
1	4.0	10.25	38.7
2	3.2	15.6	54.8
3	(i) 3.3	30.3	56.0
	(ii) 3.3	30.3	
4	4.3	19.8	29.1
5	4.2	11.9	23.8
6	3.5	9.3	14.6
7	4.5	16.7	22.3
8	4.0	25.0	50.0
9	2.5	28.0	60.0
10	3.8	52.6	60.5
11	3.5	14.3	48.5
12	2.5	4.0	8.0
13	2.5	8.0	

There is clearly a great variation in the "optimum length-increase" when comparing several pieces of ileum under similar conditions. Thus, on lengthening the gut, this figure varies between 4.0 p c and 52.6 p c, on shortening, between 8.0 p c and 60.5 p c. The cause of such extreme variation is difficult to explain, the only factor which could not be controlled was the tonus existing in the muscle itself, and changes in tonus may possibly account for it.

SUMMARY

(1) Tension/Length curves obtained from plain muscle (cat's ileum) stimulated by histamine, are very similar to those obtained from skeletal muscle stimulated electrically. The muscle was first lengthened by stages and then allowed to shorten back to its original length. The resting curve in the latter case shows smaller tensions at the greater lengths than does the resting curve during the lengthening process.

This difference is explained as being due to the viscosity of the muscle and to the stretching of the non-muscular elements of the intestine

(2) The total tensions developed on contraction appear to depend on the tensions obtaining in the relaxed condition at the various lengths

(3) There is a definite optimum length at which the maximum tension is developed by the muscle investigated. This optimum length is less on lengthening than on shortening the same muscle

(4) The optimum length varies considerably in different pieces of muscle from similar sources and under identical treatment. This appears to be due to unexplained differences of tonus

I wish to acknowledge my indebtedness to Professor Lovatt Evans, F R S, for his frequent advice during this investigation

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HISTAMINE SHOCK BY O INCHLEY

(From the Pharmacological Laboratory, Cambridge)

It is generally accepted that histamine is a direct poison to capillaries, leading to their dilatation. Dale and Richards(1), and Dale and Laidlaw(2) have shown that in histamine shock the capillaries are dilated, and that this is associated with a fall of blood-pressure and a state of collapse. Krogh(3) has shown that capillaries constantly perform active changes in calibre and suggests that there are "arteriomotor and capillariomotor systems which are able to act in opposite directions."

The contractile power of the veins had been observed as early as 1863 by Goltz(4). Rouget(5) discovered certain cells on the capillaries which have been studied by Vimtrup(6) who ascribes to them the function of constricting capillaries, though no active dilator mechanism has been described. Krogh thinks that the constrictor cells of Rouget are traceable to, and gradually merge into the plain muscle fibres of the veins and arteries. Gunn and Chevasse(7) have demonstrated the constriction of the veins by adrenaline. In the case of arterioles, dilatation when it occurs is obviously passive, the dilating force being derived from the heart. It is conceivable that, even in an excised organ, portions of capillaries, actively constricting, might squeeze their contents into adjacent capillaries, giving appearances under the microscope suggesting active dilatation. Microscopical observations, therefore, become difficult to interpret, and Krogh(8) describes, from observations on the frog, constriction occurring in certain capillaries at a time when dilatation is obtaining in adjacent ones.

Capillary dilatation is the characteristic phenomenon of histamine shock as well as of shock by other substances. Dale's view, which is generally accepted, is that histamine acts directly on the capillaries. The present experiments suggest another and simpler explanation to account for the effect, namely, that the dilatation of the capillaries is passive, and due to constriction of the veins. In a circulation through intact arteries, capillaries and veins, where a variable resistance may be imposed in any one of the three, it is difficult to draw conclusions as to which is affected in altered circulatory conditions. Each of these three factors of the circulation must be examined separately.

Experimental

I Perfusion of arteries and capillaries without veins *Perfusion of veins and capillaries without arteries* The first experiments were made by using isolated organs artificially perfused and excluding either arteries or veins. The organs of cats and rabbits were employed. The animals were killed by pithing. Cannulae were placed in the main artery and vein of the isolated organ and the organ was then freely incised so that during a perfusion through either the artery or the vein the fluid would escape, in the main, through the capillaries when the vein or artery respectively had been previously occluded. The methods of doing this necessarily varied with the organ employed. Thus in the case of the intestine the intestinal loop was opened up along the side distant from the mesentery in the liver the viscus was scarified with a hard wire brush in the limb the muscle tissue was similarly scarified after removing the fascia lata. Valves are absent or rudimentary in the mesenteric veins so that the perfusion backwards is easy under low pressure in the limbs the pressure must be increased sufficiently to render the valves in these veins incompetent. The following protocol is typical.

Cat. Loop of small intestine 15 cm. long removed. Mesenteric artery clamped. Mesenteric vein perfused with oxygenated Ringer under a pressure of 25 cm. Small intestine incised.

Number of drops in outflow in consecutive 30 sec. 17, 25, 25, 24, 27, 26, 25

After injection of 2 c.c. histamine phosphate 0.1 p.c. into tube supplying the vein the drops registered 15, 15, 14, 15, 14, 14, 13, 13, 14, 15, 14.

It is known (2) that the rabbit responds to histamine differently from the cat. It is a suggestive fact that this unusual reaction of rabbits to histamine should be associated with the fact that histamine has little constriction effect on the mesenteric veins.

As the flow through a pipe varies directly as the difference of pressure between the two ends, and inversely as the resistance in the pipe, and as in each of my experiments the pressure was kept constant, the resistance interposed as the result of histamine may be expressed as a ratio, namely, the outflow before histamine, divided by the lowest outflow obtained during histamine perfusion. These resistances for the vessels of the cat may be thus tabulated.

Organ	Vessel	Outflow before hist.	Outflow during hist.	Resistance
Intestine	Vein	25	13	2
	Artery	15	5	3
Liver	Portal vein	75	10	7.5
		6.5	1.6	4
Hind limb	Hepatic vein	12	9.5	1
	Vein	2.2	0.9	2.4
	Artery	5	1.5	3

Mautner and Pick(10) also observed marked obstruction of the hepato-portal circulation after histamine

These experiments show that histamine phosphate causes powerful constriction of both veins and arteries in the concentrations used Ringer solution adjusted to the same pH causes slight relaxation. It becomes necessary to explain why histamine causes a fall of blood-pressure with dilated capillaries, while adrenaline does the opposite. To obtain an answer to this it was suggested to me that the preparation be perfused with very dilute solutions of histamine in oxygenated Ringer, using veins and arteries alternately, to determine whether the one were more sensitive than the other in such dilutions. A preparation was made of the small intestine of the cat, one cannula was placed in the mesenteric vein supplying oxygenated Ringer at a low pressure from one funnel, another cannula was placed in the mesenteric artery supplying similar Ringer from a second funnel at a higher level. After the outflow had been provided for by the longitudinal incision in the gut, by the occlusion of one cannula, the perfusion could be obtained by the other. Perfusion by each channel was made alternately, the heights of the funnels were adjusted so that an approximately equal outflow was obtained in both cases. Perfusions with different concentrations of histamine were then made through the vein and artery. Fig 1 is the record of a typical experiment under these conditions, and it shows that the veins are more sensitive than the arteries to histamine and constrict before the arteries are appreciably affected.

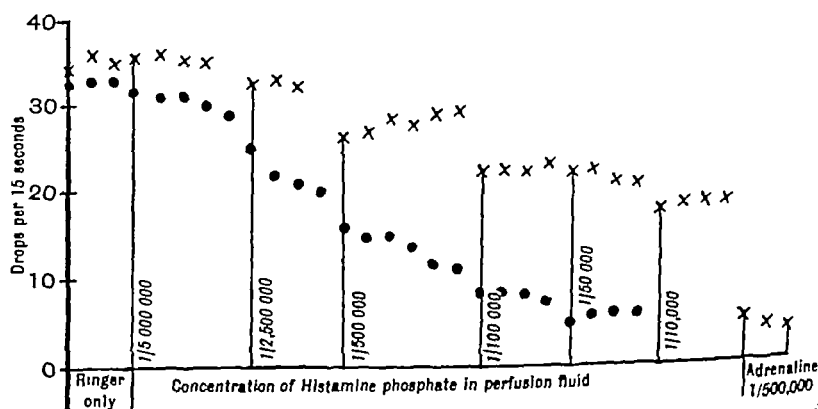


Fig 1 Cat. Excised loop of small intestine. Cannulae in vein and artery. Intestine incised. Outflow recorded in drops during intervals of 15 seconds. The perfusion fluid was supplied to the vein under a pressure of 28 cm Ringer, to the artery under 80 cm.

x = artery • = vein

II *Perfusion of arteries or veins without capillaries.* A preparation of the small intestine of the cat was made as described above, except that instead of an incision in the gut the intestine was cut away along the line of the mesenteric attachment, the vascular loops which course along the edge of the mesentery being retained in the preparation. As before, the height of the funnels was adjusted to give approximately equal outflows from either vein or artery. The following experiment is typical

Cat. Excised loop of small intestine. Cannulae in vein and artery. Small intestine removed. Outflow recorded in c.c. during intervals of 1 minute. Perfusion fluid supplied to vein under pressure of 10 cm. to artery under 51 cm.

Fluid	Outflow c.c. per min.							
	Perfusion of vein				Perfusion of artery			
Oxygenated Ringer	34	34			35	36		
Hist. phosph. 1/5 000,000	15	17			37	37		
Hist. phosph. 1/500,000	10	11	11		36	34	35	33
Adrenaline 1/500 000	6.5	7	7.5		5	6		

These experiments show, in so far as perfusion experiments are valid for the purpose, that histamine acts in the same way whether a simple arterial system is used or whether an arterio-capillary system and that histamine acts identically on a venous and on a veno-capillary system. One is forced to the conclusion that the capillaries play an insignificant part in the phenomenon.

If the isolated organ with the vessels in their natural sequence is considered there is reason to believe that histamine when perfused should here also exert effects on the veins and arteries such as I have shown in the separated vessels. If this be so, increased resistance in the veins with unaltered arterial resistance must cause (1) increased capillary pressure, (2) distention of capillaries, (3) diminished flow. If adrenaline increases the resistance in the artery more than it does that in the veins then there should follow, (1) diminished capillary pressure, (2) diminished distention of capillaries, (3) diminished flow. If the normal animal has an efficient controlling mechanism on each of these resistances, the capillary pressure and the rate of flow could each be separately adjusted to requirements.

III *Ring preparations.* These results were confirmed by using a different method. Ring preparations of the mesenteric artery and vein of the pig (from the slaughter house) were suspended in the same bath of oxygenated Ringer, simultaneous records of their movements obtained, and the effects on them of different concentrations of histamine recorded. It was found that small concentrations, such as 1/1,700,000 caused

constriction of the vein only, much stronger concentrations, such as 1/100,000 being required to affect the artery (see Fig 2) That the artery

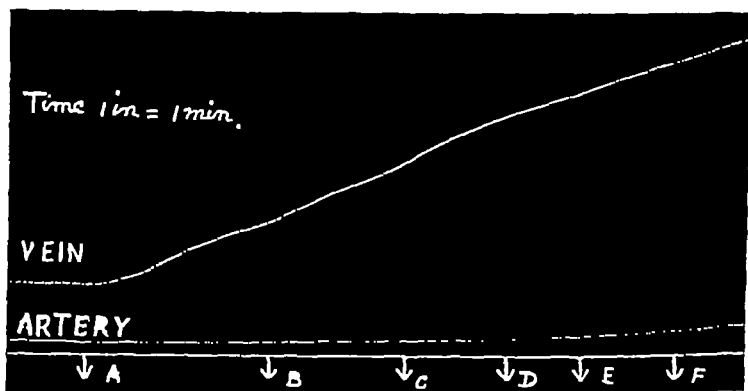


Fig 2 Isolated mesenteric artery and vein of pig suspended in a bath of oxygenated Ringer 250 c.c. capacity Histamine phosphate 0.1 p.c. was added by pipette, 20 drops from which equals 1 c.c. In the figure $A=3$, $B=6$, $C=20$, $D=20$, $E=20$, $F=20$ drops. The tensions on the thread attached to vein and artery were in the proportion of 1:3 respectively

was responsive to drugs was shown by the fact that it entered into maximal contraction on the addition of adrenaline. This method was also used with the pulmonary artery and vein of the pig. Here the smaller pulmonary veins, about 1.5 mm diameter, constrict with histamine while the corresponding arteries do not, on the other hand, the larger arteries constrict under histamine while the corresponding veins do not. Cow(9) found that the pulmonary artery may be divided into two portions, that without the lung reacting to adrenaline like other arteries, that within the lung either not affected or actually dilated. Fig 3 shows



Fig 3 Isolated pulmonary artery and vein of pig suspended as in Fig 2. In this experiment the tension on the thread attached to the ring was 0.6 gm in each case. At 4:20 drops of 0.1 p.c. histamine phosphate were added to the bath, at B, 20 drops and at C, 10 drops of adrenaline 0.1 p.c. the vein constricts but not the artery

the effect of histamine on these small vessels. Vessels of this calibre are found branching off at right angles from the main stems in the body of the lung. Fig. 4 shows the effect on larger vessels, about 5 mm

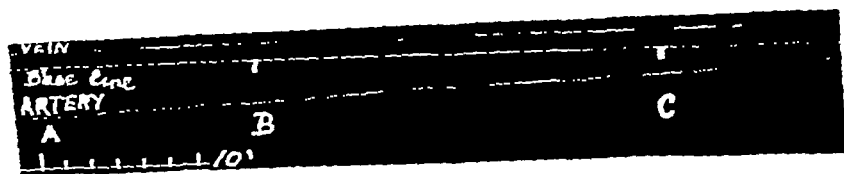


Fig. 4. (See text.)

diameter. At A, two drops of the histamine solution were added. At B two drops more, at C, eight drops. The artery contracts but not the vein.

These experiments suggest that vascular constriction under histamine is mainly venous.

IV *Experimental occlusion of veins in anaesthetised animals.* If the preceding experiments give a valid interpretation of histamine action then experimental obstruction of veins by mechanical means should also produce histamine-like effects. Occlusion of certain large veins was made by applying pressure. The veins used were (1) the inferior vena cava above the entrance of the renal veins and (2) the portal vein. Cats anaesthetised with urethane were employed and the effects were found to differ according to whether the occlusion period was short or long. Occlusion for one minute of either the inferior vena cava or of the portal vein caused a slight fall in blood-pressure with rapid recovery to normal on releasing the vein. Occlusion of the portal vein for 12 minutes caused marked fall in blood-pressure with sometimes failure of respiration. On release the blood-pressure showed only a partial recovery.

Occlusion of portal vein and vena cava together for 30 minutes caused a profound fall in blood-pressure which on release did not recover; the animal remaining permanently collapsed (Fig. 5). At this stage intravenous injection of Ringer's 50 c.c. slowly administered did not appreciably improve the condition. Adrenaline injections caused a marked but temporary rise in blood-pressure which, however, rapidly returned to the previous level of collapse. *Post mortem* the intestinal mucous membrane appeared normal in colour salmon pink rather than yellow; the surface shows the normal velvety appearance not the glassy uniform surface of oedema. The absence of congestion and oedema is

constriction of the vein only, much stronger concentrations, such as 1/100,000 being required to affect the artery (see Fig 2) That the artery

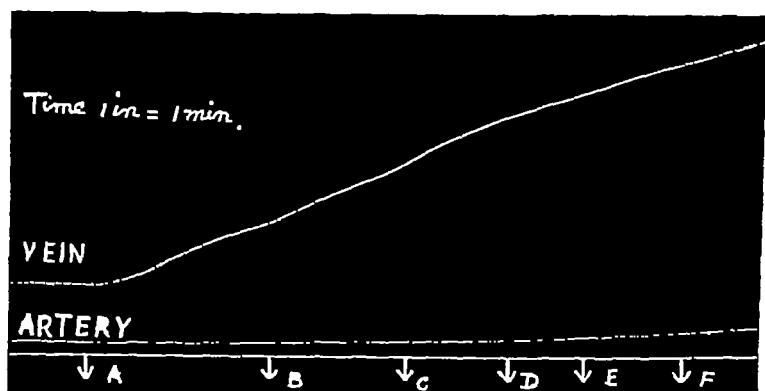


Fig 2 Isolated mesenteric artery and vein of pig suspended in a bath of oxygenated Ringer 250 c.c. capacity Histamine phosphate 0.1 p.c. was added by pipette, 20 drops from which equals 1 c.c. In the figure $A=3$, $B=6$, $C=20$, $D=20$, $E=20$, $F=20$ drops. The tensions on the thread attached to vein and artery were in the proportion of 1 : 3 respectively

was responsive to drugs was shown by the fact that it entered into maximal contraction on the addition of adrenaline. This method was also used with the pulmonary artery and vein of the pig. Here the smaller pulmonary veins, about 1.5 mm diameter, constrict with histamine while the corresponding arteries do not, on the other hand, the larger arteries constrict under histamine while the corresponding veins do not. Cow(9) found that the pulmonary artery may be divided into two portions, that without the lung reacting to adrenaline like other arteries, that within the lung either not affected or actually dilated. Fig 3 shows



Fig 3 Isolated pulmonary artery and vein of pig suspended as in Fig 2. In this experiment the tension on the thread attached to the ring was 0.6 gm. in each case. At A, 2 drops of 0.1 p.c. histamine phosphate were added to the bath, at B, 20 drops and at C, 10 drops of adrenaline 0.1 p.c. the vein constricts but not the artery

V *Painting vessels with histamine solutions* If a large vein is painted with a strong solution of histamine in an anaesthetised animal sufficient is absorbed to produce general systemic effects almost immediately, but when an artery is similarly treated only local effects are observed in the tissues supplied by it

The hind limb of the cat, anaesthetised with urethane, was placed in an oncometer. The rectum was excised and the iliac vessels exposed in the pelvis. The iliac vein and artery were consecutively painted with a 3 p.c. solution of histamine phosphate (\approx 1 p.c. hist. base). Blood-pressure and limb volume were recorded.

After either the iliac vein or the inferior vena cava was painted there was a fall in the general blood-pressure and a simultaneous moderate increase in limb volume. After painting the iliac artery an immediate marked increase in limb volume occurred but no appreciable effect on blood-pressure. After painting the vein there is a general shock with fall of blood-pressure. After painting the artery there is constriction only of the veins of the limb supplied by that vessel with resulting vasodilatation and the animal is protected in some way from shock. How this protection is produced has not been further investigated.

VI *The effect of large doses of histamine* Dale has shown that after intravenous doses of 1-2 mgrm. histamine base per kilo typical shock with failure of respiration and death occurs after 10 or 15 minutes. Such doses probably cause venous but not arterial constriction, with larger doses arterial constriction might preponderate. If this hypothesis is true then the injection of a large dose of histamine should contract arterioles as well as veins and cause a rise in blood-pressure. It has been shown that with high concentrations of histamine the arterial resistance is equal to or greater than the increased resistance in the veins, so that "bleeding into capillaries" with consequent fall in blood-pressure is to that extent diminished. Fig. 6 is a record of such an experiment. As the histamine concentration in the circulation gradually diminishes so collapse symptoms become evident. The experiment shows that the important factor in this collapse is the ratio of the arterial inflow to the venous drainaway, not the absolute amount of either factor, venous or arterial, in the resistances interposed.

Dale has described the wheal produced on the surface of the pancreas after painting with histamine solution. This phenomenon in the light of these experiments may be ascribed to constriction of venules in the presence of an adequate blood-pressure. In confirmation of this view I find that the simultaneous administration of papaverine, a drug

explained by the low blood-pressure, for if a short loop of intestine in a normal anæsthetised cat be ligatured at either end and its vein occluded

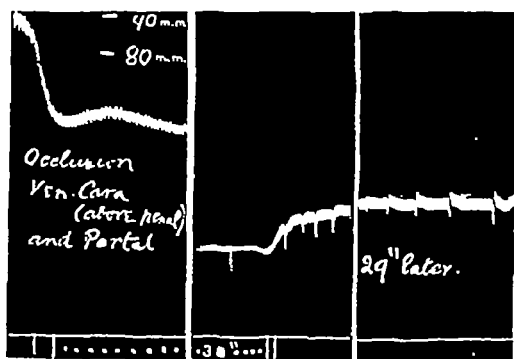


Fig 5 Cat Urethane Shows the effect on the blood pressure of occluding by pressure for 30 minutes the inf vena cava and portal vein.

for 30 minutes, no great alteration of general blood-pressure occurs, but *post mortem* the intestine is plum coloured, cedema of the mucous membrane is obvious, and punctate hæmorrhages are seen under the peritoneal surface of the gut here the sustained general blood-pressure accounts for the high capillary pressure and consequent cedema

The fall of blood-pressure during occlusion of large veins is easily explained Krogh(s) has emphasised the great capacity of distended capillaries The animal "bleeds into its own capillaries" and the effective volume of circulating fluid is so diminished as to cause the fall in blood-pressure observed The permanent low blood-pressure and collapse following after release from prolonged obstruction of great veins is also easy to explain Here resiliency of vascular tissue, which is effective in returning the pent-up blood after obstruction of short duration, becomes ineffective Such resiliency would appear to be in the capillary region and may be the effect of the tonus, either of Rouget cells or of the capillary wall itself Perhaps, under prolonged distention, such tissues may lose their tone If this be so the condition is analogous to that which obtains during digital dilatation of the anus and other sphincters Such response to abnormal and continued distention may be inherent in plain muscle Many explanations of the collapse following mechanical obstruction of veins might be offered, it deserves further investigation The action is peripheral since the same result occurs in the pithed animal under efficient artificial respiration

this fluid and 2 mgrm of histamine phosphate dissolved in 1 c c of the same fluid was injected into the arterial tubing Under these conditions the first effect of the histamine was to diminish the flow by a half, this phase was followed by a greatly increased flow the higher the perfusion pressure at the commencement of the experiment the greater was this increase

The following experiment illustrates these effects

Cat Pithed, bled. Perfusion fluid 1 part defibrinated blood, 2 parts oxygenated Ringer, adrenaline 1 in 1,000,000 Loop of bowel perfused, flow recorded in drops during consecutive 30 seconds.

- | | | | | | | | | | | | | |
|---|-----------------------------|----|----|----|---|----|----|----|----|----|----|----|
| 1 Perfusion pressure, 60 mm. of mercury | | | | | | | | | | | | |
| | Before histamine | 13 | | 12 | | 12 | | | | | | |
| | After hist. phosph. 2 mgrm. | 8 | 6 | 6 | 6 | 8 | 11 | 13 | 14 | 17 | 18 | 18 |
| 2 Perfusion pressure raised to 100 mm. of mercury | | | | | | | | | | | | |
| | Before histamine | 18 | | 18 | | 18 | | | | | | |
| | After hist. phosph. 2 mgrm. | 13 | 10 | 18 | | 32 | 49 | 53 | | | | |

It thus appears that with doses of histamine much larger than those used by Dale there is a first phase of diminished outflow followed by a second with great increase of outflow. It is reasonable to think that under the conditions of Dale's experiments, viz a perfusion pressure of 165 mm of mercury and such a small dose as 0.01 mgrm of histamine, the first phase might be less obvious, in fact only represented by the preliminary phase of increased volume, the increased outflow showing itself after a preliminary latent period.

My preceding observations suggest that this first phase is a venous obstruction. It now becomes necessary to determine if mechanical obstruction of veins, under the conditions of Dale's experiments, might lead to an increased flow of perfusion fluid.

The following experiment resembled the preceding in all respects except that a perfusion pressure of 140 mm of mercury was employed and histamine was not used, but the apparatus was arranged so that the rubber tubing on the vein cannula could be gradually occluded by a screw clamp. After a number of trials it was found that a certain degree of obstruction increased the flow. Recording drops per minute with the outflow tube fully open, the readings were 10, 10, 10. After slightly screwing down the clamp the readings remained 10, 10, 10. On further slight increase of obstruction the readings became 12, 12, 12. The clamp was now screwed down until a momentary stoppage of flow occurred, the clamp was immediately released sufficiently to allow a flow of 9, 9, 10, 10, 10. The obstruction was again increased by screwing the clamp down until the readings were 5, 5. No further alteration of the

which paralyses plain muscle, delays the onset and checks the amount of wheal formation on the skin

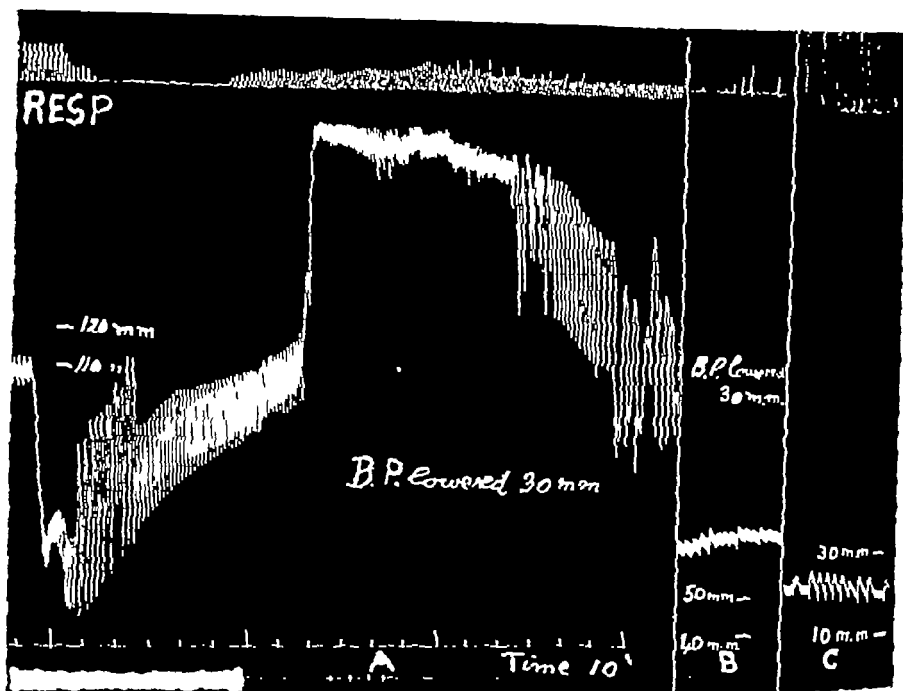


Fig 6 Cat. Urethane Blood pressure, lower curve, respiration, upper curve Shows the effect of an intravenous injection of 150 mgrm hist. phosph in 15 c.c. Ringer After a preliminary fall in blood pressure and temporary failure in respiration the blood pressure rises The condition of the animal 45 minutes later is shown in B Shortly after this artificial respiration was required. C shows the condition 1½ hours after the injection, natural respiration had returned. Fifteen minutes later respiration finally failed and the animal died, under artificial respiration, 2½ hours after the injection in a state of profound collapse

One difficulty in accepting this simple explanation of histamine action is that in the experiments of Dale and his collaborators on the limb of the cat artificially perfused with fluid containing adrenaline histamine was shown to cause increase in the limb volume and in the venous outflow

The following experiments differing in certain details from those under discussion throw some light upon them A cat was pithed and its defibrinated blood diluted with oxygenated Ringer, and adrenaline added to a concentration of 1 in 1,000,000 A loop of bowel was perfused with

this fluid and 2 mgrm of histamine phosphate dissolved in 1 c c of the same fluid was injected into the arterial tubing Under these conditions the first effect of the histamine was to diminish the flow by a half, this phase was followed by a greatly increased flow the higher the perfusion pressure at the commencement of the experiment the greater was this increase

The following experiment illustrates these effects

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- | | | | | | | | | | | | | | | | |
|---|----|----|----|----|----|----|----|----|----|----|----|--|--|--|--|
| 1 Perfusion pressure, 60 mm. of mercury | | | | | | | | | | | | | | | |
| Before histamine | 13 | | 12 | | | 12 | | | | | | | | | |
| After hist. phosph. 2 mgrm. | 8 | 6 | 6 | 6 | 8 | 11 | 13 | 14 | 17 | 18 | 18 | | | | |
|
2 Perfusion pressure raised to 100 mm. of mercury | | | | | | | | | | | | | | | |
| Before histamine | 18 | | | | | 18 | 18 | | | | | | | | |
| After hist. phosph. 2 mgrm | 13 | 10 | 18 | 32 | 40 | 53 | | | | | | | | | |

It thus appears that with doses of histamine much larger than those used by Dale there is a first phase of diminished outflow followed by a second with great increase of outflow. It is reasonable to think that under the conditions of Dale's experiments, viz a perfusion pressure of 165 mm of mercury and such a small dose as 0.01 mgrm of histamine, the first phase might be less obvious, in fact only represented by the preliminary phase of increased volume, the increased outflow showing itself after a preliminary latent period.

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clamp was made After 5 minutes the outflow was 7, 8, 10, 9, and after a further 5 minutes it became 16, 16, 19, 28, 34 From this it appears that under these special conditions mechanical obstruction of veins may lead after a sufficient latent period to increased flow, and that within limits the greater the obstruction the greater the increase of flow, but also the longer the latent period The significance of the favouring conditions, high perfusion pressure, preliminary treatment with adrenaline and the use of a corpuscular fluid needs further comment The resistance to the circulation of the blood through a system of capillaries is determined by the friction of the fluid and its contents against the capillary walls

Moderate constriction of the venous outflow, whilst placing a further and direct resistance to the outflow also by mild damming back, diminishes the internal resistance of the capillaries by dilating them It is conceivable, with a high arterial pressure under the conditions enunciated, that the venous constriction may be the means of diminishing the resistance of the veno-capillary system taken as a whole Such an effect is necessarily enhanced by the red corpuscles in the perfusion fluid and a good arterial pressure is an essential factor Further, if this explanation be valid, it implies that in any capillary there should be what may be described as two critical pressures not far apart, with the lower critical pressure as well as with all pressures below it the corpuscles are gripped by the capillary wall, with a consequent high resistance to the flow through them, with the higher critical pressure and those above it the corpuscles are free to pass easily, resulting in a low resistance to flow It follows from this that additional resistance in the veins will increase flow when the capillary pressure is in the region of these critical pressures Further, when the capillary pressure is far below or above these critical pressures, then increased resistance in the veins will diminish flow These considerations account for the erratic behaviour in perfusion experiments, where in the same preparation sometimes the Dale phenomenon is obtained, while at other times the opposite effect occurs

It is a pleasure to acknowledge the advice and help of Dr W E Dixon throughout this research

CONCLUSIONS

1 Histamine in low concentrations constricts the veins, leaving the arteries unaffected Such constriction leads to passive dilatation of capillaries

2 Long continued occlusion of great veins leads to permanent relaxation of capillaries

3 Evidence is produced to show that histamine shock is best explained by venous constriction

The expenses of this research were partly defrayed by a grant from the British Medical Association.

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THE ACTION OF ALCOHOL UPON CONDUCTION IN THE AURICLE OF THE TORTOISE

By ALBIN SELIŠKAR¹

(From the Department of Pharmacology, University College, London)

THE writer has determined the influence of alcohol upon the rate of conduction of the wave of excitation in isolated strips of the auricle of the tortoise. The method employed has been described in detail in a previous paper⁽¹⁾. The strip of auricle was suspended in a bath of Ringer's fluid, and was stimulated electrically at one end with periodic induction shocks, and the electrical and mechanical responses were measured at two or more points along the strip. The measurements obtained in a single typical experiment are shown in Table I.

TABLE I The action of ethyl alcohol (1 Mol.) on the rate of conduction in an isolated strip of tortoise auricle

(Distance between recording electrodes *a-b*, 24 mm. Frequency of contractions, 19 per min.)

Time since excision of the auricle h m	Fluid	Time of action in mins	Interval between electrical changes at electrodes <i>a-b</i> in secs	Rate of conduction in mm per sec	Height of mechanical response in mm
27 00	Normal Ringer	20	0.25	96	20
27 11	Ringer + Ethyl alcohol 1 Mol.	1	0.276	87	4
27 32	Normal Ringer	10	—	—	—
27 32	Ringer + Ethyl alcohol 1 Mol.	2	0.41	59	2
27 58	Normal Ringer	13	—	—	—
27 58	Ringer + Ethyl alcohol 1 Mol.	3	1.15	21	0.5
28 24	Normal Ringer	10	0.22	110	23

The results of a series of experiments are shown in Table II. In this table the results are expressed as percentages of normal action. The figures in Table II show that the alcohols depress the force of contraction far more powerfully than they depress the rate of conduction. The rates of action on conduction of ethyl alcohol (1 Mol. and 0.5 Mol.), *n*-butyl

¹ Fellow of the Rockefeller Foundation.

TABLE II. The action of various alcohols upon the rate of conduction and force of contraction of excised strips of tortoise auricles.

(Contractions of less than 0.5 mm. are shown as 0)

No of experiment	Drug	Molecular concentration	Time of action in mins.	Rate of conduction (Normal=100)	Height of mechanical contraction (Normal=100)
XX	Ethyl alcohol	1.0	1	87	17
XX	"	"	1	94	13
XX	"	"	2	56	8
XX	"	"	3	21	2
XX	"	"	4	No electrical variation	0
X	"	"	4	89	11
XVI	"	"	3	76	8
XVII	"	"	5	56	8
XXIII	"	0.5	2	62	20
XXIII	"	"	5	44	0
XXIII	n Butyl alcohol	0.02	1	98	0
XXIII	"	"	3	93	0
XXIII	"	"	5	86	0
XXIII	"	0.03	3	87	0
XXIII	"	"	4	89	0
XXIV	n Amyl alcohol	0.02	1	68	0
XXIV	"	"	3	51	0

alcohol (0.02 Mol), and *n*-amyl alcohol (0.02 Mol), are shown in Fig. 1. These graphs give, of course, only approximate values and no mathematical treatment can be attempted. The figure shows, however, that an equal effect is produced on conduction by 0.5 Mol ethyl alcohol and 0.02 Mol *n*-amyl alcohol. This gives a ratio of activity of 1 to 25, which is similar to the ratio between ethyl alcohol and iso-amyl alcohol found by Vernon(2), who measured their action in reducing the amplitude of contraction of the tortoise ventricle.

Some of the experiments indicated a progressively increasing diminution of conduction of the wave of excitation in the auricle after alcohol, but further observations designed to test this point, although not conclusive, gave evidence more in favour of a uniform decrease in conduction through the whole length of the auricle. Fig. 2 shows some of the results obtained.

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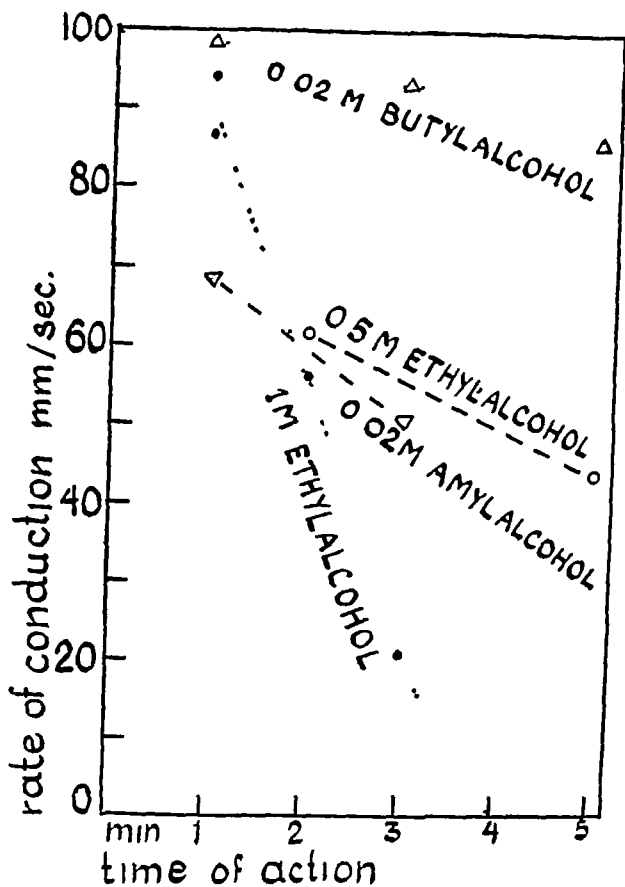


Fig 1 The action of ethyl, *n* butyl and *n* amyl alcohols on the rate of conduction in the tortoise auricle

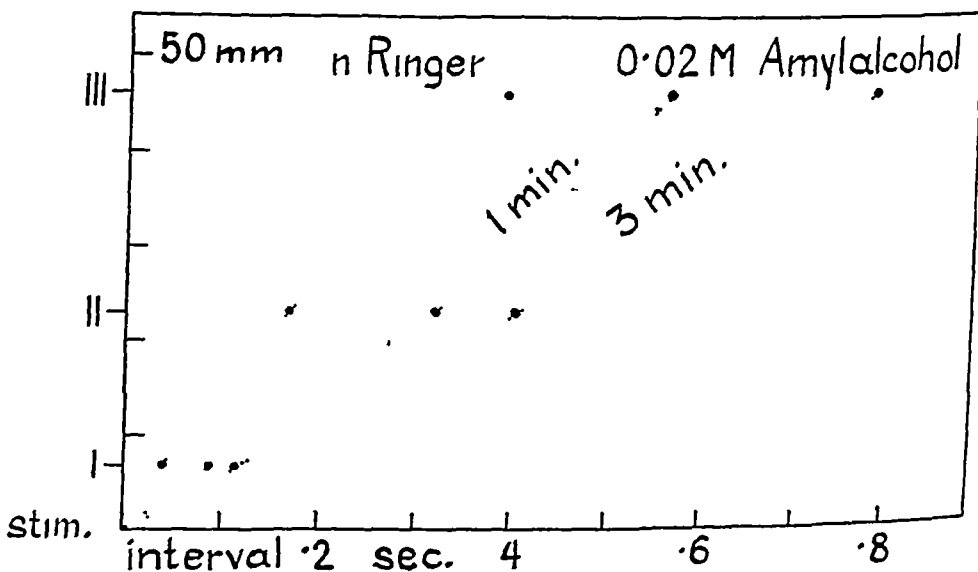


Fig 2 The action of *n* amyl alcohol on the rate of conduction in the tortoise auricle. Ordinates distance in mm from stimulating electrodes. The figures I II and III indicate the position of the recording electrodes. Abscissae time at which the wave of excitation reaches the electrodes.

ON THE INFLUENCE OF HYDROGEN ION CONCENTRATION AND OF ANOXÆMIA UPON THE HEART VOLUME

BY H GREMELS (*Rochefeller Fellow*) AND E H. STARLING

(*From the Department of Physiology and Biochemistry,
University College, London*)

IN their early work on the heart-lung preparation Jerusalem and Starling⁽¹⁾ found that administration of carbon dioxide caused a dilatation of the heart. Patterson⁽²⁾, continuing this investigation, found that adrenaline exercised an antagonistic action to the carbon dioxide, and that CO₂ caused an increase in the heart volume accompanied by a decrease in the minute output. This decrease was small with moderate percentages of carbon dioxide (8 p c), but a larger effect was produced with higher percentages. We thought it worth while to take up this question again, making a more detailed examination of the pH and the gases of the blood.

Methods. All the experiments have been carried out on dogs using the heart-lung preparation. The output of the left ventricle was determined by measuring the amount of blood flowing out on the venous side of the resistance. This did not include the coronary flow, which was determined by introducing a Morawitz cannula into the coronary sinus. According to Markwalder and Starling⁽³⁾ this drains three-fifths of the coronary blood. The heart volume was recorded on a kymograph, using Palmer's large piston-recorder connected with the cardiometer by air transmission. In those experiments in which the coronary flow was measured, much care had to be taken that the edge of the cardiometer did not obstruct the coronary sinus. A cannula introduced in the inferior vena cava was connected with a water manometer. It seemed to us that the most natural way of altering the pH of the blood was to add greater or less amounts of CO₂ to the air used for artificial respiration. To eliminate the slowing effect of CO₂ on the heart rate we drove the heart at a constant rate with weak faradic stimulation of the sinoauricular node. The hydrogen-ion concentration was estimated colorimetrically by the method of Dale and Evans⁽⁴⁾. Determinations of the

carbon dioxide content of the blood were made partly by Van Slyke's volumetric method, and partly with the new manometric apparatus designed by Van Slyke and Neill(5) For the estimation of oxygen saturation the Haldane method was used The gas mixtures employed for the ventilation of the lungs were made up in Douglas bags of a capacity of 27 cubic feet (760 litres) In order to keep the blood well oxygenated we added 20 p c of oxygen to the mixture for most experiments To determine the influence of anoxæmia upon the heart volume a mixture of air and nitrogen or pure nitrogen itself was supplied

Influence of carbon dioxide on the heart volume It is well known that in the heart-lung preparation we have a condition of acapnia This must be ascribed partly to the excessive ventilation of the lungs, partly to the disproportion of the amount of blood and the CO_2 -producing tissues Owing to this the $p\text{H}$ of the blood is considerably higher than in the normal animal and varies from 7.50 to 7.93 A typical experiment is shown in Table I and Fig. 1 It will be seen that the $p\text{H}$ shows a wide variation according to the percentage of CO_2 in the respiratory mixture, we did not estimate the actual CO_2 content of the alveolar air The lower the $p\text{H}$ the greater is the volume of the heart With 4.6 p c CO_2 in the air breathed the $p\text{H}$ goes down from 7.93 to 7.40, while at the same time the heart volume increases 12 c c (The volume of the ventricles in our experiments ranged from 83 to 102 c c) The dilatation affects both the systolic and the diastolic volumes Using 7.7 p c CO_2 in the air breathed we obtained a greater effect, the heart volume increasing by 25 c c The output of the left ventricle was diminished with this percentage of CO_2 , while 4.6 p c had no influence on the output Similar results were obtained in every other experiment The dilatation is dependent on the decrease in the $p\text{H}$ of the blood produced by the higher concentration of CO_2 in the air breathed The absolute effect varies from dog to dog, but is constant for any one preparation

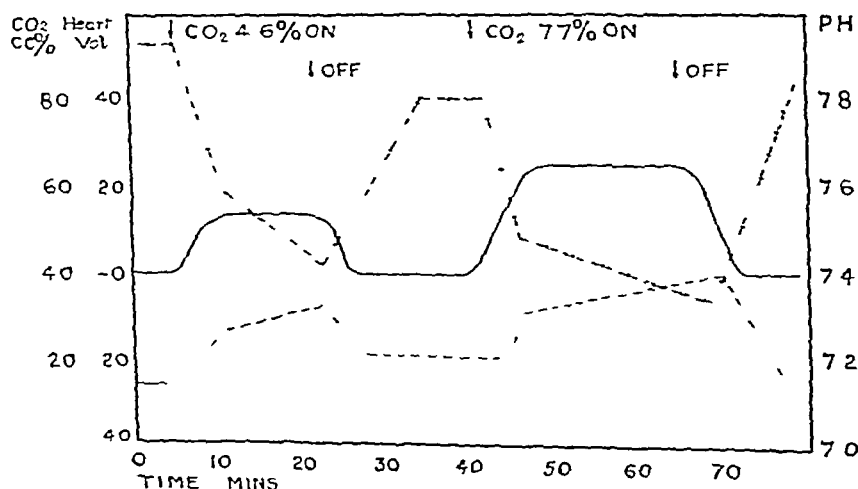
To determine whether other acids produce a similar effect we added hydrochloric or lactic acid to the blood It will be seen from Table I that the addition of 20 c c $n/10$ HCl lowered the $p\text{H}$ from 7.90 to 7.58 and caused an increase in the heart volume of 8 c c The same lowering of the $p\text{H}$ when produced by carbonic acid had a greater effect on the heart volume, increasing it by 12 c c The explanation of this will be discussed later

The influence of the $p\text{H}$ on the coronary flow Hilton and Eichholtz(6) demonstrated the effect of $p\text{H}$ on the coronary vessels in the heart-lung preparation They found that the addition of CO_2 to the air breathed

TABLE I. Effects of changes of pH on the heart volume

Ventilation	Time hours and min.	O ₂ satura- tion per cent.	CO ₂ cc. per 100 c.c.	pH of blood	In- crease in heart volume c.c.	Sys- temic output c.c. per min.	Cor- onary flow c.c. per min.	Venous pres- sure in c.c. mm. Hg	Arterial pressure mm. Hg	Heart rate beats per min.	Temp C.
Air	12.44-12.53	94.47	13.1	7.93	0	300	50	5.0	90	162	37.5
Air CO ₂ 4.6 p.c.	12.54-12.57	93.00	21.7	7.59	12	300	60	—	90	—	—
"	12.57-1.09	93.00	31.4	7.40	12	300	65	5.5	90	—	—
"	1.11-1.25	94.00	20.0	7.80	0	300	50	—	90	—	—
Air CO ₂ 7.7 p.c.	1.26-1.30	93.35	28.1	7.48	25	294	75	6.0	90	—	—
"	1.30-1.50	93.00	40.1	7.32	25	288	80	6.0	90	—	—
"	1.51-2.17	95.00	19.0	7.85	0	294	53	5.5	90	—	—
N ₂	2.17-2.23	40.00	14.7	7.89	15	252	100	7.0	88	—	—
"	2.29	19.00	12.7	7.93	10	210	180	12.5	70	—	—
Air	2.29-2.43	85.00	13.6	7.90	0	294	94	10.0	85	—	—
"	2.43-2.47	91.50	11.8	7.69	0	294	50	9.0	88	—	—
"	2.47-2.52	93.75	10.0	7.58	8	278	70	9.0	88	—	—

* n/10 HCl, 10 c.c.

Fig. 1 ——— = heart volume ——— = CO₂ - - - - - = pH

caused dilatation of the coronary vessels and an increase in the coronary flow. We are able to confirm this from our experiments, as can be seen from Table I.

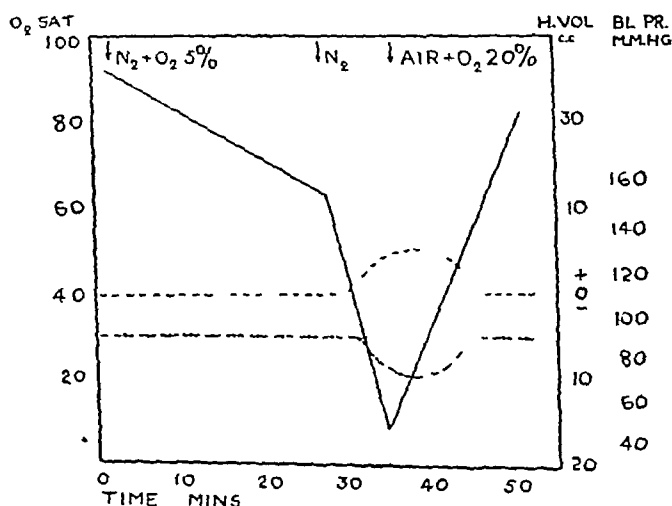
Time relations between the application of CO₂, the change in pH and the heart volume. The heart volume responds immediately to a change in pH of the blood. Usually the dilatation reaches its maximum in two to three minutes after the administration of CO₂. After this the heart volume remains steady so long as the same ventilation mixture is used. The effects of removal of CO₂ show the same time relations, the heart volume

returning to the same point as before the administration of the CO_2 mixture. In some cases the heart volume does not completely recover, but this is probably dependent on the general condition of the heart. When the heart is in good condition it always recovers completely, on the other hand, a heart in bad condition does not recover completely, as can be seen from the increasing venous pressure and coronary flow. The carbon dioxide content and the pH of the blood take considerably longer in reaching a steady level. The maximum change is obtained in 10 minutes, but after this they remain constant. The fall in the carbon dioxide content and in the pH of the blood, after the removal of the CO_2 mixture, occurs more rapidly. When the pH is changed by the addition of hydrochloric acid in place of CO_2 , the heart behaves in a similar manner but with a difference in degree. It can be seen from Table I that when 10 c.c. of $n/10$ HCl were added the pH had dropped after 4 minutes from 7.90 to 7.69, but no dilatation occurred. The addition of a further 10 c.c. of the same acid lowered the pH to 7.58 and caused a dilatation of 8 c.c. This interesting difference between the physiological effect of carbon dioxide and of another acid agrees with the findings of other authors working on different problems. Jacobs(7), in his study of the action of carbon dioxide on infusoria, was able to show that this acid has a greater effect than any other acid. The greater penetrating power of CO_2 for the cell membrane is generally regarded as the cause of this phenomenon. Similarly, Hartree and Hill(8) have concluded that CO_2 penetrates the cell walls of a skeletal muscle and causes a lowering of pH more quickly than other stronger acids.

Influence of anoxæmia In several experiments we examined the influence of anoxæmia upon the heart volume. For this purpose pure nitrogen was used for the respiration, and when a slower development of anoxæmia was required this was preceded by nitrogen containing an admixture of 5 p.c. of oxygen. The results of a typical experiment are shown in Table II and Fig. 2. We observe that the degree of oxygen saturation has no influence on the heart volume until it has dropped to about 40 p.c. At this point dilatation occurs and continues to increase as the oxygen saturation falls to 8.5 p.c. But under these circumstances the heart soon begins to fail, as is shown by a fall of blood-pressure and irregularity of the heart beat when the oxygen saturation falls lower than 10 p.c. A glance at the column giving the figures for the coronary flow shows a progressive increase, first remarked by Markwalder and Starling. The behaviour of the heart during anoxæmia shows that increase in coronary flow (with a coronary cannula inserted) has no

TABLE II. Effect of anoxæmia on the heart volume.

Ventilation	Time hours and mins.	O ₂ satura- tion per cent.	CO c.c. per 100 c.c.	pH of blood	In- crease in heart volume c.c.	Sys- temic output c.c. per min.	Cor- onary flow c.c. per min.	Venous pres- sure in c.c. mm. H ₂ O	Arterial pressure mm. Hg.	Heart rate beats per min.	Temp C.
Air	11 30-12.15	93.00	12.73	7.93	0	306	20.0	6.0	86	190	37.0
N ₂ O ₂ 5 p.c.	12 16-12 19	87.60	12.03	7.94	0	306	22.4	6.0	86	—	—
"	12 23	85.00	9.64	7.96	0	303	24.0	6.5	86	—	—
"	12 28	78.80	9.60	7.96	0	300	25.0	6.5	86	—	—
"	12 35	71.06	9.60	7.96	0	294	27.6	7.0	86	—	—
N ₂	12 42-12.43	48.00	9.60	7.96	+ 2.2	276	38.0	10.0	86	—	—
"	12 45	26.80	9.60	7.97	+ 3.0	240	60.0	15.0	80	—	—
"	12 47	8.50	9.60	7.98	+ 5.0	190	120.0	21.0	85	—	—
Air O ₂ 20 p.c.	12 48-1 6	82.50	9.60	7.96	0	294	29.6	14.5	85	—	—
Air CO ₂ 8 p.c.	1 9-1 11	89.00	12.31	7.74	+ 6.0	286	37.2	12.0	85	—	—
"	1 13	90.05	15.15	7.53	+ 8.0	282	39.0	12.0	85	—	—
"	1 18	90.00	32.20	7.50	+ 12.0	270	43.8	12.0	85	—	—
"	1 25	90.05	47.07	7.39	+ 14.0	270	54.0	13.0	85	—	—
Air O ₂ 20 p.c.	1 26-1 29	90.00	31.91	7.53	+ 3.0	280	42.0	13.0	85	—	—
"	1 35	91.00	14.10	7.90	+ 1.5	286	40.0	13.0	85	—	—

Fig 2 ——— = O₂ sat. - - - - - = heart vol. = bl. press.

demonstrable influence on the heart volume. In all the experiments in which we produced want of oxygen we see a large increase in the coronary circulation without any change in the heart volume. Only when the oxygen saturation falls below 40 p.c. does the heart dilate. On the other hand, administration of CO₂ causes a smaller effect on the coronary circulation but an immediate dilatation of the heart. We conclude, therefore, that it is the action on the heart muscle itself which causes alteration of the heart volume.

The pH during anoxæmia shows a slight increase when the oxygen saturation reaches a low level, while the carbon dioxide content of the blood remains steady or increases slightly. It has been shown by Hasselbalch(9), Christiansen, Douglas and Haldane(10), A V Hill(11) and Parsons(12) that oxyhæmoglobin reacts more acid than reduced hæmoglobin. It may be that this is the cause for the shifting of the pH to the alkaline side during anoxæmia. Our findings with regard to the relationship between anoxæmia and heart volume do not agree with those of Takeuchi(13). His method of measuring the heart volume, namely, by means of a cinematographic record of the heart's surface, cannot be as accurate as the cardiometer. Takeuchi finds that the most pronounced influence occurs when the oxygen saturation drops from 100 p c to 80 p c, a less marked influence being observed in the more severe degrees of anoxæmia. Takeuchi used the whole animal, whereas we are dealing with only the isolated heart, which is independent of any nervous influence. In his experiments we notice the remarkable effect of oxygen want on the heart volume in the higher stages of oxygen saturation. We are inclined to ascribe his results to the influence of the anoxæmia, not on the heart muscle itself, but on the central nervous system. As it is well known, the central nervous system is sensitive to even slight degrees of anoxæmia, with stimulation of the vagal and vasomotor centres as a result. This stimulation of the vagi slows the heart beat and, in order to keep the output of the heart constant, the volume of blood thrown out per beat has to be greater than before and the heart therefore dilates. In this way we can explain Takeuchi's results. Only in the heart-lung preparation can we observe the pure influence of any one factor, such as anoxæmia, on the heart muscle.

The influence of the pH of the blood on the heart rate. We know from the work carried out in this laboratory that CO_2 has a slowing effect on the heart rate. In order to avoid this we drove the heart at a constant rate in most of our experiments. In some of them this procedure was omitted in order that we might observe the influence of CO_2 on the heart rate. As may be seen from Table III, the heart rate falls from 144 to 124, with a drop in pH of the blood from 7.67 to 7.38, brought about by administration of CO_2 . Throughout these experiments the temperature was maintained absolutely constant. Andrus(14) measured the conduction time in the heart muscle under varying hydrogen-ion concentrations and found that the rate of transmission was lowered as the pH of the fluid used to feed the heart was diminished. On the other hand, on raising the pH he found that the conduction time was quicker than

TABLE III. Effect of changes of pH on the heart rate

Ventilation	Time hours and mins.	O ₂ - satura- tion per cent.	CO c.c. per 100 c.c.	pH of blood	In- crease in heart volume c.c.	Sys- temic output c.c. per min.	Cor- onary flow c.c. per min.	Venous pres- sure in r.v.c. cm. H ₂ O	Arterial pressure mm. Hg	Heart rate beats per min.	Temp. C.
Air	12 20	88.50	24.04	7.67	0	294	—	8.5	81	144	35.8
Air CO ₂ 6-45 p.c.	12 28-12 30	89.00	23.90	7.50	+10	288	—	9.0	81	136	35.8
"	12 37	88.50	35.08	7.38	+10	282	—	9.0	81	124	35.8
"	12 48	88.00	39.63	7.38	+10	282	—	9.0	81	124	35.8
Air	12 54-12 57	89.00	30.33	7.54	0	282	—	9.5	81	142	35.8
"	1 1	89.00	20.89	7.74	0	282	—	9.5	81	144	35.8
Air CO ₂ 6-45 p.c.	1 2-1 14	89.00	40.34	7.38	+10	282	—	11.0	81	124	35.8
Air	1 15-1 21	89.00	22.60	7.74	0	282	—	11.0	81	144	35.8

before. He gives the following explanation: the rhythm and the conduction time in the cardiac tissue are normally determined solely or in large part by the pH of the surrounding fluid. It is considered that the excitatory process in the heart, as in other irritable tissues, represents a disturbance of ionic equilibrium between cell contents and tissue fluid, and that conduction is due to the direct stimulation of adjacent tissue by the disturbance so developed at the point originally excited.

Anoxæmia does not affect the heart rate until the oxygen-saturation reaches a very low percentage. When or soon after this point is reached heart block frequently occurs.

Discussion

From the foregoing experiments we see that alteration of the hydrogen-ion concentration to the acid side causes a dilatation of the heart. The degree of the dilatation depends on the strength of carbon dioxide used in the air mixture for ventilation in order to change the pH. We find that the heart volume attains its maximum within 3 minutes after beginning the ventilation with CO₂, whereas the pH of the blood continues to fall for about 10 minutes. The explanation lies probably in the buffering mechanism between blood and tissue. When the CO₂ content of the blood rises to such an extent that the buffering power of the blood itself becomes insufficient, there is a passage of tissue-alkali from tissues to blood. On the other hand, CO₂ enters the heart muscle-cells, and both these factors are responsible for the change in the actual reaction of the heart muscle which brings about its dilatation.

Our experiments do not explain why the equilibrium in the heart muscle is established earlier than in the blood. This can be done only by an investigation of the relative influence exerted by the blood and the tissue respectively on the buffering process.

The behaviour of the heart volume during anoxæmia shows that,

up to a considerable degree of desaturation of the blood, the heart still takes up sufficient oxygen for its needs, partly by increasing the flow through the coronary vessels, partly by increasing the coefficient of utilisation, as was pointed out by Hilton and Eichholtz. Thus it shows no signs of dilatation until the oxygen saturation falls to about 40 p c. But a pronounced dilatation, often associated with heart block, sets in with oxygen saturations lower than 10 p c.

CONCLUSIONS

1 The isolated mammalian heart dilates when the hydrogen-ion concentration of the perfusing blood increases.

2 The degree of dilatation is dependent on the rise of hydrogen-ion concentration.

3 Anoxæmia has no influence upon the heart volume until the oxygen saturation falls to about 40 p c.

4 The dilating influence of anoxæmia is most pronounced below 10 p c oxygen saturation. At the same time signs of heart failure occur.

5 During the lower degrees of anoxæmia the pH becomes shifted to the alkaline side by about 0.02.

We wish to express our thanks to Mr R. A. Nash, who kindly assisted in performing the experiments.

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THE CONDUCTION OF A NERVOUS IMPULSE IN A NARCOTISED REGION OF NERVE

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UNTIL challenged recently by Kato⁽¹⁾ in Japan and Forbes⁽²⁾ in America, it was taken as an established fact that, when subjected to narcosis or other depressing influence, a nerve conducted with a decrement, or to quote Keith Lucas⁽³⁾ "The nervous impulse undergoes a change which increases with the distance travelled through the narcotised nerve. This change we may, if we will, speak of as a reduction in strength or intensity, but we ought to have clearly before our minds that the actual change observed is reduction of the ability to be conducted through narcotised nerve." These conclusions are based on a long series of experiments starting with those of Grunhagen⁽⁴⁾ in 1872 and culminating in the work of the Verworn school in Germany and of Lucas and Adrian in this country. But Kato and his co-workers have put forward experiments from which they conclude that nerves in a state of depressed conduction do not conduct with a decrement.

Kato deals first with the type of experiment in which different lengths of nerve are narcotised at a uniform rate. It is generally stated that the time necessary to suspend conduction through the narcotised area becomes shorter as the length of nerve is increased. Kato fails to confirm this result and finds that in comparing different lengths of the two sciatics of the Japanese toad, conduction fails at the same time whatever the lengths narcotised, provided only that these exceed 6 mm. His controls with equal lengths of nerve show variations of as much as 20 p.c., this error seems to be unaccounted for in his experimental results, which otherwise show a very close agreement. In dealing with the effect of narcotics on very short lengths of nerve, he proves that the shortest or "limit length" of nerve that can be narcotised adequately is about 6 mm. and that in shorter lengths than this the results are complicated by spread of the stimulus to normal nerve and diffusion of the external Ringer solution into the narcotic, thereby lowering the depth of narcosis.

He objects to the usual method of testing for the spread of the stimulating current by ligating the nerve at different distances from the

electrodes, since the effects of the ligature hinder the spread of the current to the nerve beyond. He proves this point by cutting or burning the nerve and shows that an electric stimulus may be effective owing to spreading when cutting or burning has no effect. But in order to obtain quantitative results on current spread, he uses a method depending on the determination of the conduction time from kymograph records of the latent period, and the accuracy of this method is not great.

In other experiments he finds that the length of narcotised nerve makes no difference to the size of the contraction, to the size of the negative variation or to the least interval for muscular summation. The increase in this interval found by Adrian and Lucas(5) he ascribes to a uniform slowing of the rate of recovery from the refractory state.

Kato's work has been criticised by Ishikawa(6), who has repeated many of the experiments and obtained results contrary to those of Kato, he considers that the stimuli used by Kato were much too strong. On the other hand, Forbes and Davis(2) have measured the size of the action current in the peroneal nerve of the cat in alcohol narcosis, they state, in agreement with Kato, that there is a uniform decrease in the size of the action current along the nerve during narcosis, and therefore there must be no decrement in conduction.

Investigation of current spread

In view of the fact that Kato has criticised work in which due attention has not been paid to current spread, it was thought advisable, before proceeding further with work on decrement, to make a quantitative investigation of the spread from the electrodes in use. Lucas quoted some figures for his glass fluid electrodes(7), where the nerve passed through a small hole, and also for his electrodes for an uncut nerve(8). In the latter case he required an increase of threshold strength to 50 times the normal value to get a spread of 2 mm. The slot fluid electrodes used in the present work were of a similar nature to these, but I had reason to suppose that the spread was slightly greater. Lucas' results were obtained by ligaturing the nerve, and as Kato has criticised this, it was thought better to use a method depending on the rate of conduction which was measured by the plan adopted by Lucas(9) in his experiments on alcohol.

A frog's sciatic-gastrocnemius preparation was placed in a chamber similar to that shown in Fig. 1. There were four slot electrodes *A*, *B*, *C* and *D* and the preparation was in Ringer solution. Descending break shocks were used as stimuli, two induction coils were arranged so that

the first could stimulate at all four electrodes, and the second only at electrode *A*. The strength of stimulus could be varied by resistance boxes

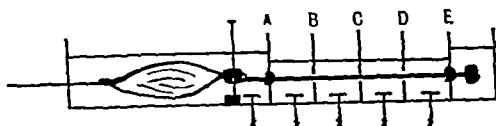


Fig 1

in the primary circuits and the stimuli were delivered by a Lucas pendulum. In carrying out an experiment, the least interval for muscular summation was found at electrode *A*, using a second stimulus of ten times the threshold strength and varying the strength of the first stimulus. This variation was usually found to make no difference to the value of the least interval after the strength had reached three times the threshold, if the first stimulus was greater than 15 or 20 times the threshold the least interval was found to be a little smaller. Probably for stimuli under three times the threshold the response was not wholly maximal and when the stimulus was made strong the response was not always simple. Following Lucas' method⁽⁹⁾ for measuring the rate of conduction, the first stimulus was sent in at each of the other electrodes and at each electrode the strength was varied between three and fifteen times the threshold strengths. It was assumed that in each case the true rate of conduction could be calculated when the first stimulus was three times the threshold and that any decrease in the least interval for greater strengths was due to current spread.

The method of calculation is shown, taking figures from an actual experiment, and denoting the threshold strength as θ

If a secs = least interval at *A* when first stimulus = 3θ

„ b „ = „ *B* „ = 3θ

„ c „ = „ *B* „ = $p\theta$

and $AB = x$ cm

Then in $b - a$ secs impulse covers x cm

in $c - a$ „ „ $\frac{x(c-a)}{b-a}$ cm

for a stimulus $p\theta$ the spread = $x - \frac{x(c-a)}{b-a}$ cm

Temp 11°C $a = 0.00348$ sec

$b = 0.00516$ sec

$c = 0.00508$ sec when first stimulus = 10θ

$x = 2.5$ cm

$$\begin{aligned}\text{spread} &= 2.5 - \frac{2.5(0.00508 - 0.00348)}{0.00516 - 0.00348} \text{ cm} \\ &= 0.12 \text{ cm}\end{aligned}$$

The mean result for five experiments gave a spread of 1.6 mm for a stimulus of ten times the threshold value (14 determinations) and a spread of 2.1 mm for a stimulus of fifteen times the threshold (9 determinations). A few observations were made with platinum electrodes, they were too few to quote the result, but they showed clearly that the condition of the nerve is important. The nerve must not be allowed to dry, nor must the moisture on it be excessive, for in either of these cases the escape may vary a great deal and lead to inexplicable results, this conclusion is confirmed in experiments in which a gas is passed over the nerve the gas must be saturated with water vapour and the flow of gas must not be so great that it tends to dry the nerve.

While dealing with current spread it may be as well to emphasise the fact again that with a bull frog's nerve such as was used by Kato, the escape is much greater than with a green frog's, Erlanger and Gasser⁽¹⁰⁾ quote actual figures showing that whereas with a green frog's nerve there is little or no escape, with that of a bull frog there may be as much as 10 mm escape. From observations in the course of experiments it was concluded that with slot electrodes the local condition of the nerve plays some part, but never to the extent of causing a spread of over 3 mm for a stimulus ten times the threshold strength, a good stretch of nerve fitting the slot well will occasion little or no spread.

Conduction in alcohol

Having satisfied myself that slot electrodes were eminently suitable for experiments in which current spread was particularly to be avoided, I proceeded to investigate the state of a nerve in narcosis, using a solution of alcohol as a narcotic and the same chamber as that shown in Fig. 1. The slots were 8 mm apart in the earlier experiments, and in the later they were altered so that *A* and *B* were 11.5 mm apart and the others 8 mm, this was done because the stimuli from *B* were apt to take effect on normal nerve, as shown in control current spread experiments. But even with the longer length of *AB* it was impossible entirely to overcome this. It was due probably not to a spread of the stimulus but to a diffusion of the Ringer solution in the muscle chamber tending to lower the degree of narcosis at the peripheral end of the narcotised stretch. The slots at *A* and *E* were plugged with vaseline and the muscle and the central end of the nerve were in Ringer solution during the

whole of the experiment, the part of the nerve between *A* and *E* could be covered with Ringer or alcohol solution at will. The stimulating apparatus was the same as that described on p. 307 except that the stimuli could both be effective at all the electrodes so that the least interval could be measured at each electrode.

In most experiments the procedure was to record the threshold, the least interval for muscular summation and recovery curve, where possible, at each of the slots *B*, *C* and *D*. The threshold and least interval at *A* were also measured at the beginning and from time to time during the experiment, if these remained constant, it was a good indication that the alcohol was not leaking at *A*. As soon as the nerve had reached a steady condition the Ringer solution was replaced by alcohol dissolved in Ringer. Various strengths were used between 5 p.c. and 10 p.c., a 5 p.c. solution caused fairly rapid loss of conductivity and was useful for determining the final values of the least interval and the time taken for the nerve to become inexcitable. A 6 p.c. or 7.5 p.c. solution was used most often since one of these would generally cause the nerve to reach a steady state in which recovery curves could be recorded, these may not be quite comparable with those obtained when the least interval is changing, but very often with alcohol the changes are very rapid, and it is impossible to get any reliable curves. An experiment was only taken into account if the thresholds kept more or less together at the start and later increased in smooth curves, if the threshold at one electrode was obviously different from the others, or the increase was erratic, then it was assumed that for some reason the nerve was being unequally narcotised and the results were discounted.

Fig. 2 shows the results of an experiment in graphical form. The upper curves give the least interval for muscular summation at the three electrodes, and the lower ones give the threshold values. The observed values of the least interval are shown, but the thresholds are given as $\frac{\text{normal value before narcosis}}{\text{observed value}}$ and the normal value is expressed as unity. All the curves show the preliminary fall which is associated with the application of narcotics, then at about 4 minutes after the alcohol is put in all the curves show a rise. At electrode *B*, 1.15 cm. from the normal part of the nerve, the threshold rises, showing that the nerve is becoming narcotised, but the least interval only shows a very small rise and then remains steady, probably indicating that the impulses quickly reach a less narcotised portion of the nerve after leaving *B*. At *C* and *D* the thresholds and least intervals both rise and in the experiment shown in Fig. 1, conduction failed completely from *C* and *D*, but it did not fail

at the same time, there was no response at *D* in 20 minutes and at *C* in 29 minutes

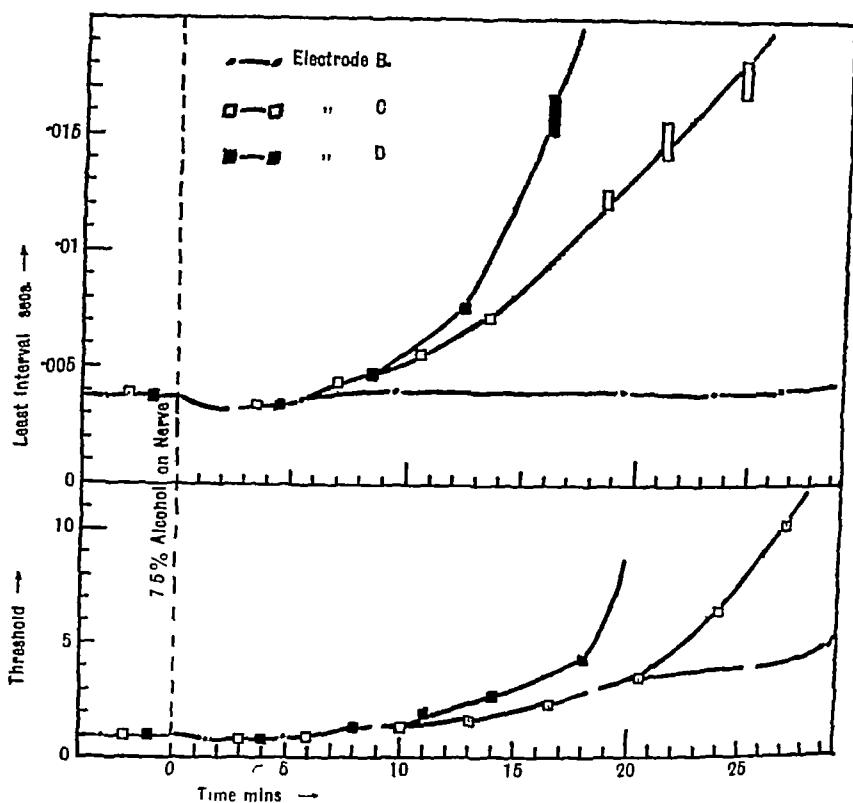


Fig 2

TABLE I.

Exp	Temp °C	Normal least interval sec.	Strength of alcohol p c.	Time of reading min.	Threshold at time of reading			Least interval at time of reading		
					<i>B</i>	<i>C</i>	<i>D</i>	<i>B</i> sec.	<i>C</i> sec.	<i>D</i> sec.
1	12.8	0029	7.5	12	2.6	3.5	4.0	0035	0075	01
2	13.8	0028	5.0	15	1.75	1.75	1.85	0032	005	0059
3	14.6	0028	7.5	13	2.5	3.1	2.2	0031	0068	012
4	14.0	0027	5.0	15	2.8	2.7	2.6	0027	0031	0033
5	14.5	0026	6.0	20	1.8	1.7	1.6	0025	0036	0038
6	14.5	003	7.5	20	2.7	2.9	3.8	003	007	014
7	11.8	0038	7.5	15	2.4	4.0	∞	0059	0072	just failed
8	11.0	0039	7.5	20	3.0	3.4	∞	004	0135	"
9	11.0	0032	7.5	20	2.6	2.1	4.2	0036	0084	0104

In Table I the results of nine experiments are shown. In constructing the table I have thought it best to give the readings at a time in the

course of the experiment when the least interval is showing a definite rise, rather than take a series of experiments in which conduction failed completely at the upper electrodes and to quote the times of the failures. At this later stage a much larger current strength is required and the possibilities of current spread and abnormal response in the nerve are greatly increased. In the table the least interval at *B* only serves to emphasise the fact that at the end of the chamber the narcosis is not so deep and it is easy for the impulse to get to more or less normal nerve. But at *C* and *D* it is unlikely, if the impulses do spread, that they would escape past the next slot. If this is so then we can safely compare the columns of the least interval at *C* and *D*, and in every case we find the least interval at *D* greater than at *C*, in the two cases given in which conduction has failed at *D*, conduction does not fail at *C* until 8 and 9 minutes later respectively. The thresholds serve to show that there is no great variation, or else the threshold at the more central electrode was the greatest. Before leaving this side of the experiments, I would draw attention to the fact that in cases where conduction failed completely I never recorded a least interval greater than about 0.015 second. This has a bearing on the rate of recovery, as was shown in an earlier paper (11).

As well as recording the least interval, in many experiments I attempted to measure the recovery curves at some time during the narcosis. In our present state of knowledge, two alternatives are offered to explain the increase of the least interval when a nerve is placed in a medium which impairs conduction. The first is that the nerve conducts with a decrement and has an unaltered rate of recovery and the other is that the recovery rate is lengthened. The former is supported by the work of Lucas (9) and the present writer (12) which showed that the rate of recovery is unaltered in alcohol narcosis and asphyxia, the latter by Kato's evidence against the possibility of decremental conduction. According to Kato, since the second impulse suffers no decrement in its passage, the increase in the interval must be due to a slowing of the recovery process. If this is so we should expect to find a slowing of the relative as well as of the absolute refractory period, and the time relations of the recovery curve would be prolonged, as they are when the nerve is cooled.

The recovery was generally recorded when the nerve had reached a steady state, as by this means it was sometimes possible to get a complete curve. The curves obtained were of two kinds, as shown in Fig 3 *a* and *b*. These curves are probably only modifications of the same state, Fig 3 *a* is the one usually explained as the result of decremental conduction,

the vertical part being supposed to indicate the degree of decrement and the horizontal part coinciding with the original curve and indicating that the rate of recovery is unaltered. In Fig 3 *b* there is a more gradual

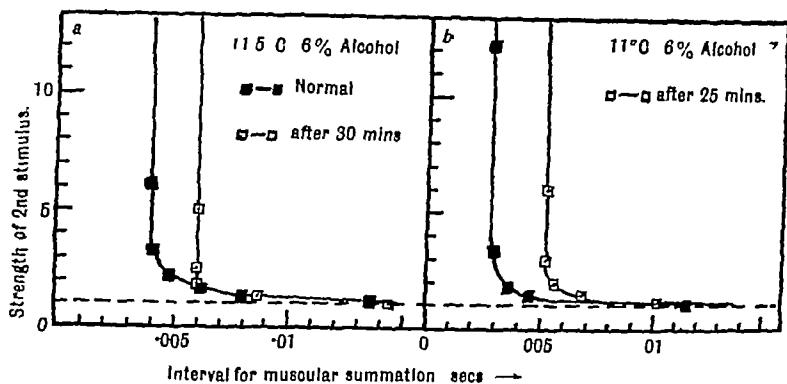


Fig 3

change from the vertical to the horizontal, but there is no doubt that the curve finally coincides with the normal curve and the total refractory period is in no way prolonged. The final curve in Fig 3 *b* bears a general resemblance to the normal curve, if the rate of recovery were altered uniformly for all stages of recovery, the curve would be very much more gradual in its change from vertical to horizontal and it would not reach the threshold value until an appreciable time after the normal curve does so. There was absolutely no indication of these things in any of the curves that were recorded and it is justifiable to conclude that in narcosis the prolongation of the least interval is due either to conduction with a decrement or else to a change in the rate of recovery which increases the absolute refractory period but leaves the total recovery time unchanged.

Kato observes that if the narcotised nerve is stimulated in several places then the muscle responses all get smaller together. As far as can be made out from his book, his pupil Minami used "maximal stimuli" at an outside electrode, using different lengths of two nerves. I carried out some experiments on the size of the mechanical response, using the chamber previously described. The stimuli were chosen so as to bear the same relation to the threshold at each electrode, on the grounds that if there is no decrement the conduction should fail for the same strength of stimuli at the same moment, this method maintains the same current strength all the time and obviates current spread. Fig 4 shows a reproduction of the actual responses from an experiment, 10 p.c. alcohol was

used and the stimuli were six times the normal threshold at each electrode, the stimuli were given every half-minute at first and later at

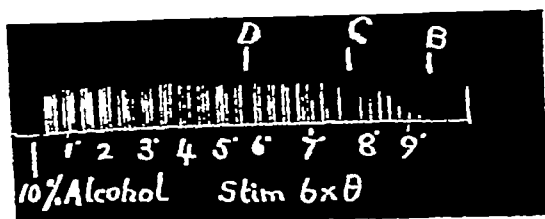


Fig 4.

every quarter-minute. It will be seen that the responses from *D* became smaller while those at *C* are still of normal size and the responses fail from *D* at $5\frac{1}{2}$ minutes, from *C* at $7\frac{1}{2}$ minutes, and from *B* at $9\frac{1}{2}$ minutes.

Arising out of the same problem, attempts were made to carry out experiments using the electric responses as an indication of conduction. The nerve was arranged on three pairs of platinum stimulating electrodes and monophasic responses were led off at one end by non-polarisable electrodes of the AgCl type. The nerve was in an airtight chamber through which air or alcohol vapour could be drawn. Owing to the difficulty of taking electric responses in a liquid, a vapour had to be used and this was obtained by drawing air through a solution of alcohol in water. The responses were led off to a string galvanometer and the string movements were recorded on cinematograph film. The stimulating apparatus was as before. The object of the experiments was to narcotise the nerve and to send in two stimuli at each electrode to see whether there were intervals which gave a double response from the near electrode but only a single one from the far one. According to Kato, if a double response is obtained from one electrode there should be a double one from all three. There are indications in several experiments that it is possible to get a single response from the far one and double responses for the same interval from the two nearer ones, a definite result of this nature is expressed in Table II.

TABLE II.

Interval between stimuli sec.	Nature of response		
	Distance travelled by impulses		
	5 cm.	18 cm.	29 cm.
0.040	Double	Single	Single
0.048	Double	Double	Single

These results are suggestive but not conclusive, for the experiments were difficult to carry out and on the whole unsatisfactory

All these experiments suffer from the use of alcohol as a narcotising agent. Alcohol is commonly made use of in any experiments of this nature, but the results from its use are not ideal. It probably penetrates the nerve fairly quickly and hindrance to its entry at any part of the nerve is quickly reflected in irregularities in the excitability at different points. An effect of alcohol which makes its use troublesome is the relation between strength of alcohol and loss of conductivity. With a weak solution the conduction is frequently never completely lost during the average time of an experiment, the contraction merely gets very small and the summation is poor so that it becomes almost impossible to measure the least interval accurately, though from the results that can be obtained it does not rise very much. On the other hand, with a strong solution the actual rise of the least interval may be very rapid, but the same strength of alcohol often varies in its action considerably from nerve to nerve.

Conduction in chloral hydrate

Owing to the unsatisfactory nature of alcohol, its use was abandoned and a solution of chloral hydrate was tried in its place, this gave remarkably good results from the start. Several strengths of chloral hydrate were used and it was found that the results bore a very fair relation to the strengths used. Table III gives a summary of the experiments in which

TABLE III.

Exp	Temp °C	Strength of chloral hydrate p.c	Thresholds together for mins.	Time of divergence of least interval		Conduction fails	
				D from C mins.	C from B mins.	D mins.	C mins.
10	18	4	10	3½	7	20	21½
11	18	4	15	9	11	22½	26
12	18	4	18	10	13	20	23½
13	17	4	7 app	6	11	<27	29
14	18.5	3	18	11	13	29	34
15	18	3	16	9	—	40	52
16	18	2	25	22	22	52	60

the threshold and least interval were followed, from this it will be seen that conduction failed at the more distant electrodes at an average time of about 22 minutes for a 4 p.c. solution, 34 minutes for a 3 p.c. and 52 minutes for a 2 p.c., while at the middle electrodes the respective times are 25, 43 and 60 minutes. There was never any doubt about the conduction from the more distant electrode failing first, in Exp. 10 there

is only a $1\frac{1}{4}$ minutes difference, which does not look very much, but in the actual experiment when conduction failed at *D* (Fig 1) there was still quite a decided contraction from *C*. This was so in every experiment and there was absolutely no indication of conduction failing at the same moment from both electrodes. A consideration of the thresholds and least intervals shows that the thresholds remain together for an appreciably longer time than the least intervals (cf column 4 with columns 5 and 6 in Table III). The significance of the thresholds remaining together points to equal narcotisation of the nerve, while the divergence of the least intervals points to a decrement in conduction. A typical result from one experiment is shown in Fig 5 taken from Exp 12, the

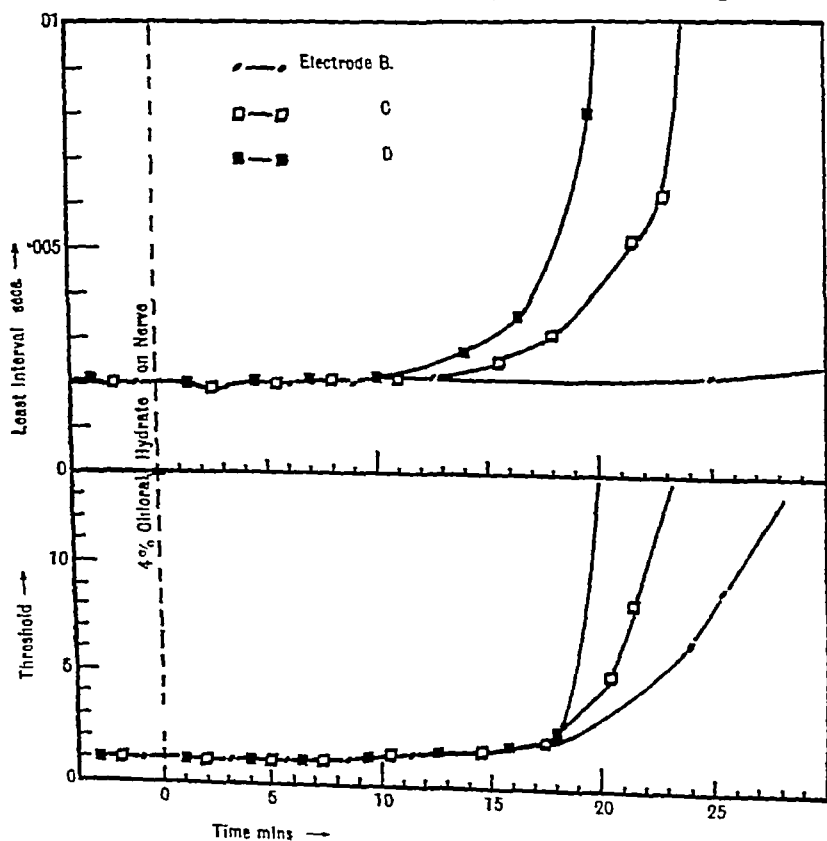


Fig 5

thresholds are absolutely together for 18 minutes, whereas the least interval at *D* diverges from that at *C* in 10 minutes and at *C* from that

at *B* in 13 minutes, the curves are very smooth and it is easy to read off the figures that have just been given. It is difficult to get exact values for the threshold or least interval just before conduction fails, but the actual time of failure is in most cases correct to ± 15 seconds.

With the chloral hydrate, as with alcohol, attempts were made to follow out the recovery. There was sometimes a tendency for a super-normal phase to appear, especially with a 3 p.c. or 2 p.c. solution that took a long time to act, but with a 4 p.c. solution it was possible to obtain some very satisfactory curves. One of these is shown in Fig. 6,

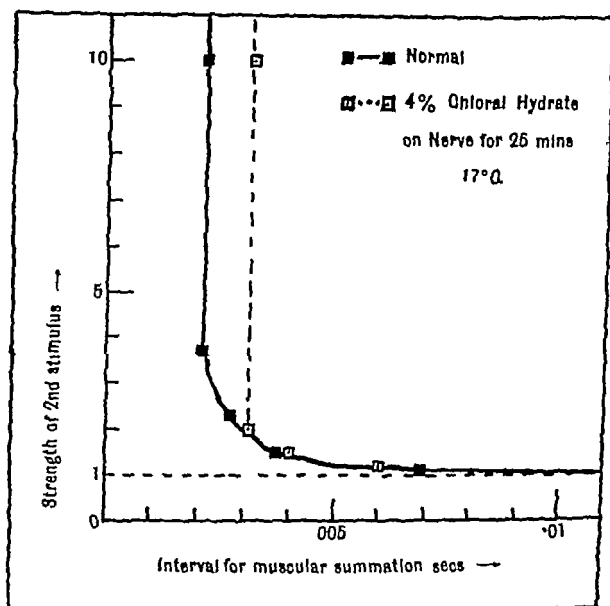


Fig. 6

the curve was obtained quickly, and control measurements were made on the threshold throughout, so that the curve should be a reliable picture of the state of the nerve. There is no doubt that the curve from the narcotised nerve is made up of two parts, one a vertical part independent of the normal curve and the other identical with the normal curve, the first expressing the degree of decrement and the second the unaltered rate of recovery. Some further experiments were carried out in which the lower limit of the vertical part of the curve was measured, the least interval was found with a strength of second stimulus ten times the threshold and then the least strength was found that would give

summation at this interval This value should lie on the normal recovery curve, and in practice it was always found to lie either on the normal curve, or just below it In the latter case it was probably due to a slight supernormal phase These experiments serve to confirm the more uncertain results with alcohol and in themselves they give a very satisfactory picture of the happenings in a narcotised nerve

If the narcotic merely alters the whole state of the nerve without causing a decrement, a change in the recovery curve might be expected This would be manifest in the shape of the curve, but the continuity of the curve would be unaffected and, more important still, the curves from different electrodes would be identical In Fig 3 *b* the curve for the alcohol narcotised nerve can be regarded as a change of shape, but I was never able to obtain two such curves from adjacent electrodes, therefore it can probably be put down to some local condition of the nerve caused by the unequal action of the alcohol The curves obtained during the use of chloral hydrate never showed this change of shape, they were always discontinuous, the shape of the lower part of the curve being unaltered from that of the normal curve, and the curves differed progressively at the different electrodes

It might be possible that the progressive change down the nerve was due to histological differences which made some parts more permeable than others Thus, for instance, the region at which the nerve sends out large branches to the thigh muscles might be more permeable But during the course of a considerable number of experiments, nerves of all sizes have been used and it has never seemingly made any difference if the electrodes have been directly on the part where the branches are given off It might also be argued that the peripheral end of the nerve in the narcotic is near Ringer solution and is thus different from the central end, but in most of the experiments the central end was also in Ringer solution (cf Fig 1), so that there should be no difference in the two ends of the nerve

In studying the question of a decrement in conduction in a nerve, it may be necessary to differentiate between the use of one stimulus and of two or more stimuli, in the first case, where we are dealing with only one impulse, it may well be that a decrement does not occur until just before the final loss of conduction Conduction does fail earlier when a greater length of nerve is narcotised, but the difference in the times required is small But when two stimuli are used the second impulse has to face not only a narcotised stretch of nerve, but also an incompletely recovered nerve and the two may combine to cause a decrement

in conduction In nearly all the experiments in the foregoing paper two stimuli were used and the conclusions reached are that a narcotised nerve recovers from the passage of an impulse at the same rate as a normal nerve, just before the nerve fails to conduct, a single impulse certainly suffers a decrement in conduction, whereas an impulse in incompletely recovered nerve is conducted with a decrement at a much earlier period

SUMMARY

Preliminary observations were made to test the amount of current spread from slot electrodes used to stimulate a frog's nerve The spread was found to amount to not more than 2.1 mm for a stimulus 15 times the threshold value

Using the same electrodes, nerves were narcotised with alcohol and the course of the least interval followed at successive points along the nerve, where possible the recovery curves were also recorded at the different electrodes The results indicate that there is conduction with a decrement and no lengthening of the recovery rate But alcohol proved not to be entirely satisfactory as a narcotic, so that further experiments were done with chloral hydrate This gave much more definite results which amply confirmed the previous results with alcohol

The conclusions on the whole do not confirm the recent work of Kato, but they are in agreement with the work of Lucas and others who maintain that at some stage of narcosis a nerve conducts with a decrement and keeps throughout an unaltered recovery rate

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THE OSMOTIC PRESSURE OF THE PROTEINS OF HUMAN SERUM AND PLASMA

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IN 1896 Starling(1) showed by direct measurement that the serum proteins exerted an osmotic pressure of 41 to 56 cm H_2O when put up in bell-shaped osmometers against 1.03 p.c. NaCl. In a later paper(2) this fact was confirmed, and by concentrating serum by filtration through a porous cell soaked in gelatin and allowing the concentrated serum to dialyse against the protein-free filtrate, it was shown that there was a rough proportionality between the protein content of the serum and the observed final pressure. For example, a serum containing 6.5 p.c. protein exerted a colloidal pressure of 38 cm H_2O , and one containing 11.2 p.c. protein a pressure of 61 cm H_2O , giving pressures of 36 and 34 cm H_2O respectively per gram protein nitrogen p.c.¹ In 1907 Moore and Roaf(3) measured the osmotic pressure of various colloidal solutions including serum. Their figures for pig's serum showed this to be 24 cm H_2O at 12° C and 42 cm H_2O at 18° C, the protein content of the serum being 7.97 p.c. The magnitude of the pressure discovered by Starling was thus confirmed. Recently Govaerts(4) has described a method based on the apparatus of Moore and Roaf in which the pressure of the non-diffusible constituents is exerted on a bubble of air in a capillary tube, the final pressure of which is read by connecting it to a water manometer in the manner described by Krogh(5). Govaerts' apparatus can be used with as little as 1.5 c.c. serum or even less. The semi-permeable membrane used is "cellophane," which Govaerts states is permeable to NaCl and glucose and retains gum arabic and all proteins precipitable by trichloroacetic acid. He finds normal human serum to exert an osmotic pressure of 35 to 40 cm H_2O or a mean of 29 cm per gram of protein nitrogen p.c. In clinical cases of severe oedema the pressure varies from 12 to 22 cm, giving a value of 13 to 19 cm per gram of protein nitrogen p.c. On the other hand, in pure hypertension

¹ Corresponding to pressures of 5.8 and 5.5 cm. H_2O respectively per gram protein p.c.

cases the pressure was found to be raised (40–70 cm), giving a value of 32 to 34 cm H_2O when calculated as the pressure exerted by 1 gm protein nitrogen p c Govaerts states that the low values found in cases of oedema result from two factors the dilution of the blood and the diminution of the pressure per gm protein p c, the reverse factors acting inversely in the group of hypertension cases Govaerts gives no figures for the effect of simple dilution on the osmotic pressure of normal serum proteins, and it would seem desirable that this should be investigated before it is justifiable to invoke a second factor in the interpretation of the low values found in some of his clinical groups This relationship has been determined and the results will be reported in this paper

Method and technique An apparatus identical in its essential components with that used by Govaerts has been employed I am indebted

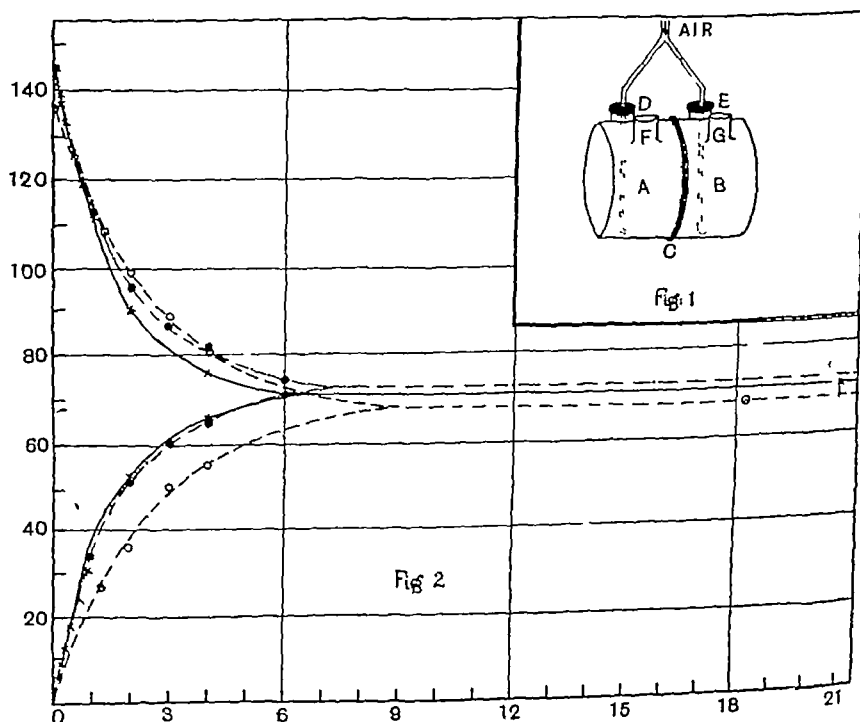


Fig 1 Apparatus for testing permeability of "cellophane" membrane

Fig 2 Equilibrium attainment curves between equivalent solutions of $NaCl$ *—*, $CaCl_2$ —○—, $CO(NH_2)_2$ —●—, and distilled water Ordinate = Milli-equivalents Abscissa = Time in hours.

to him for the supply of "cellophane" with which these experiments have been carried out. The membrane has been tested for its permeability to various substances in the crystalloid state and to serum proteins and hæmoglobin by making use of the apparatus shown in Fig 1. It consists of two symmetrical and cylindrical glass cups *A* and *B*. The everted lips of these cups are ground flat, and between them is placed a circular disc of cellophane. The two vessels are clamped securely together, their respective cavities being then separated merely by the cellophane membrane. Four short tubulures, *D*, *E*, *F*, *G*, two from the top of each vessel, communicate with the exterior. Through two of them, *D* and *E*, pass thin glass tubes which on the one hand reach to the bottoms of the cylinders and on the other are connected to an air pressure pump, so that a slow stream of bubbles may keep the solutions on either side of the membrane efficiently mixed. The capacity of each vessel is 45 c c and the surface area of one side of the intervening membrane is 15 sq cm, giving a diffusion area of 0.33 sq cm per c c of fluid as compared to 0.56 sq cm in the case of the osmometers used in the present investigation. When laked blood was placed in *B* and distilled water in *A*, no protein was detected in the latter at the end of 24 hours. The rate of attainment of equilibrium between equivalent solutions of NaCl, CaCl_2 and $\text{CO}(\text{NH}_2)_2$ and distilled water at room temperature, 19° to 20° C, is shown in Fig 2. *A* was filled with distilled water and an equal volume of the solution in question placed in *B*. 2 c c were removed for analysis at intervals through the tubulures *F* and *G*. It will be seen from the figure that equilibrium is established in the case of these three bodies within 12 hours.

The disposition of the osmometers has been slightly altered from that adopted by Govaerts in order that the whole apparatus may be submerged in a thermostat. A sectional drawing of one osmometer under this arrangement is shown in Fig 3. The whole technique is carried out with strictly aseptic precautions. If these are not taken organisms readily grow in the serum and in the dialysate, both of

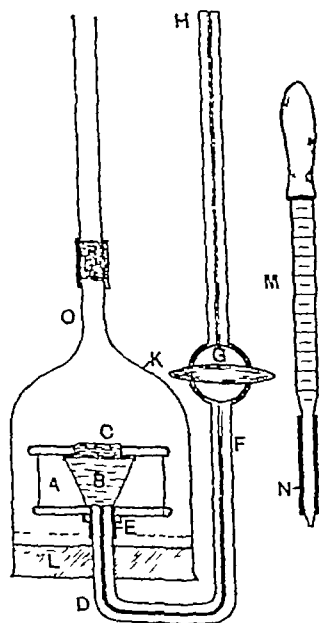


Fig 3 Osmometer

which become cloudy, and this infection is accompanied by a progressive fall in the osmotic pressure of the proteins

The rubber washers, cellophane discs and Ringer's solution are autoclaved for 45 minutes at $1\frac{1}{2}$ atmospheres and the osmometers and pipettes and centrifuge tubes dry sterilised at 130°C for 1 hour. The inside of the capsule, the perforated copper disc, and the rim around the periphery of the dialysate are coated with a layer of sterile paraffin wax, as recommended by Govaerts, so that in no place do the fluids come into contact with the metal of the osmometer. The capsule when completed holds 1 c.c., and before filling it with serum it is tested for leaks by placing a little Ringer on the surface of the membrane and raising the internal pressure. Serum or plasma is then pipetted into the capsule, the capillary *D* inserted to such an extent that the fluid passes round to the level shown in the figure at *F*, the tap *G* being open. The top of the capillary *H* is then connected to the mercury level (see Fig. 4) and the meniscus of the serum exposed to a pressure approximately equivalent to that which experience has shown to be the expected final osmotic pressure value. The capillary is then pushed further into the capsule, so as to bring the meniscus back to its original level, and sealed in position with paraffin wax. The tap *G* is closed, the osmometer cup placed over the capsule on to the thin rubber bung, and about 5 c.c. of Ringer placed on the surface of the bung by means of a curved pipette. The osmometer is now immersed in the thermostat and allowed to reach the bath temperature. This usually takes about half an hour and can be assumed to have occurred when re-exposure of the meniscus *F* to the same counter pressure as before causes no change in its position. 0.5 c.c. Ringer's fluid is now dropped on to the surface of the membrane by means of the special pipette shown at *M*, the point being guarded by a rubber tube *N* in order that it may not become contaminated by accidental contact with the inside of the tube *O* of the osmometer cup. A long tube is connected to the end of this, a piece of wool soaked in Ringer lightly plugs the junction, and the whole apparatus is more deeply immersed in the bath into the position shown in Fig. 4. Twenty-four hours later the pressure of the air confined between the meniscus and the tap is determined by observing the meniscus by means of a microscope fitted with a 3" objective and micrometer eyepiece, opening the tap and bringing the meniscus to its original level by adjusting the mercury cup (Fig. 4). The reading of the water manometer corrected for the difference in level between the meniscus and the surface of the dialysate, and for the capillarity of the tube *D* (Fig. 3), which in my

apparatus amounted to about 1 cm H_2O , gives the osmotic pressure of the non-diffusible constituents of the serum. The osmometer is then dismantled, the capillary tube taken out *without* opening the tap *G*, and

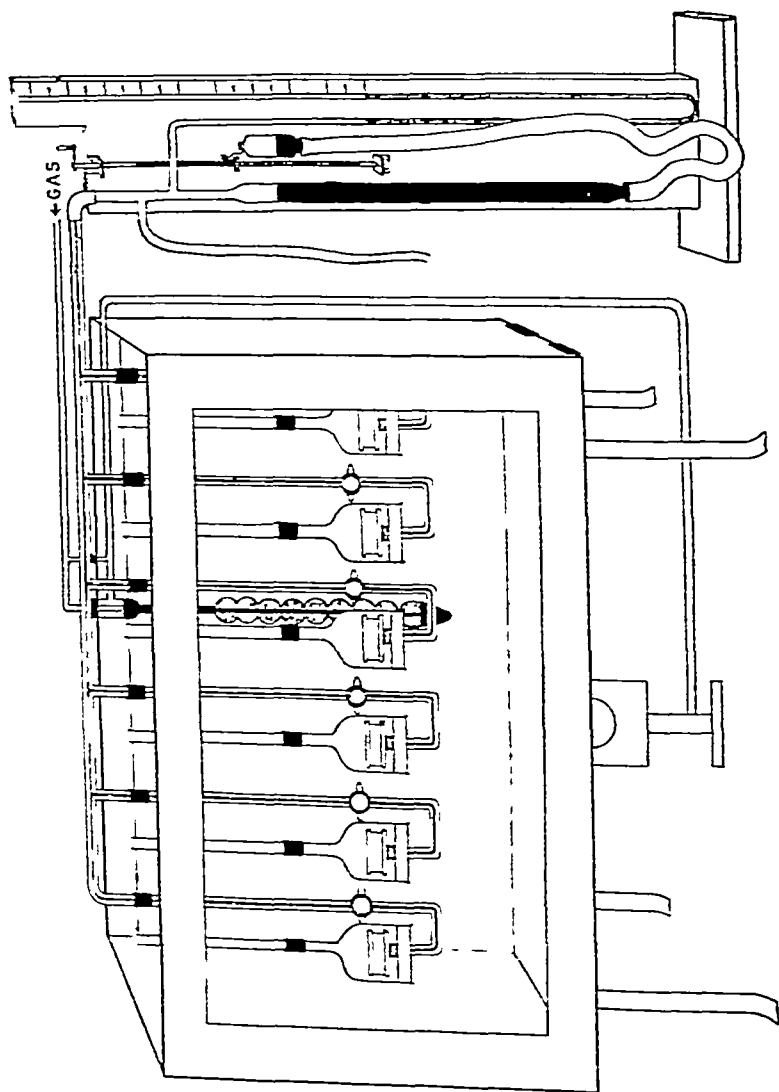


Fig. 1 Osmometers in thermostat

the serum removed for the determination of the total and non-protein nitrogen by means of a Kjeldahl apparatus

Six similar osmometers have been used and they have been set up

in parallel and connected to the mercury level by means of a capillary tree as shown in Fig 4

Experimental results The degree of accuracy of the method is shown in Table I

TABLE I Cat's serum 1 c.c. placed in each osmometer Dialysate=0.5 c.c. Ringer without bicarbonate Set up 29 xi 24 at 4 p.m. under gross counter pressure of 30 cm H₂O Temperature=17.4° C

Time	No of osmo meter	Temp of bath	Gross pressure reading cm H ₂ O	Level diff cm	Capil larity cm H ₂ O	Osmotic pres sure	Mean osmotic pres sure	Max deviation from mean	Max percent age error from mean
1 xi 24									
10 30 a.m.	1	17.4	42.2	-0.9	-1.2	40.1			
"	2	"	40.0	-0.3	-1.3	38.4			
"	3	"	41.0	-0.9	-1.3	38.8			
"	4	"	43.3	-1.0	-1.6	40.7			
"	5	"	41.0	-0.5	-1.3	39.2			
							39.3	1.4	±3.6
1 xi 24									
5 p.m.	1	18.0	42.3	-0.9	-1.2	40.2			
"	2	"	40.6	-0.3	-1.3	39.0			
"	3	"	40.3	-0.9	-1.3	38.1			
"	4	"	41.4	-1.0	-1.6	38.8			
"	5	"	41.5	-0.5	-1.3	39.7			

TABLE II. Human serum 10 c.c. venous blood drawn 9 xi 24. Repeated 1½ hours later Serum A and B set up under initial pressure of 36 cm H₂O against 0.5 c.c. Ringer (RSB) at 6.15 p.m. in duplicate Temp =18.4° C.

	Time	Temp ° C	Osmotic pressure cm H ₂ O	Mean value	Protein nitrogen mgrm p.c.	Osmotic pressure per grm protein nitrogen p.c.
A	10 xi 24					
	1 30 p.m.	18.4	34.4			
	"	"	35.8			
	4 30 p.m.	"	34.2			
	"	"	35.2	35.0	1150	30.5
B	6 p.m.	"	35.1			
	"	"	35.7			
	10 xi 24					
	1 30 p.m.	18.4	36.2			
	"	"	36.9			
B	4.30 p.m.	"	36.0			
	"	"	37.4	36.7	1150	31.8
	6 p.m.	"	36.3			
	"	"	37.4			

The Ringer's fluids used in these experiments had the following composition

$$\left. \begin{array}{ll} \text{NaCl} & 0.85 \text{ p.c.} \\ \text{KCl} & 0.042 \\ \text{CaCl}_2 & 0.024 \\ \text{NaHCO}_3 & 0.02 \end{array} \right\} = \text{RCB}$$

on the one hand, and the same fluid without the bicarbonate (= RSB) on the other. The osmometers were set up invariably in triplicate or duplicate. Two control experiments on human serum are given in Table II.

No appreciable difference between serum and plasma proteins could be detected in the osmotic pressure values, calculated per gram protein nitrogen p.c., for the blood of the same subject drawn at different times, as the following table shows.

TABLE III. *A* = Human serum readings. 29 v 25, 12 c.c. blood drawn from vein. Serum set up against 0.5 c.c. RCB against initial pressure of 30 cm. H₂O at temperature of 15° C in triplicate, at 6 p.m. *B* = Human plasma readings. 3 v 25, 11 c.c. blood drawn from vein into 0.1 c.c. saturated neutral potassium oxalate. Plasma set up as serum *A* against initial pressure of 35 cm. H₂O at 15° C in duplicate at 7 p.m.

	Time	Temp. ° C.	Osmotic pressure cm. H ₂ O	Mean value	Protein nitrogen mgm. p.c.	Osmotic pressure per gram. protein nitrogen p.c.
<i>A</i>	30 v 25					
	1 30 p.m.	15.0	36.9			
	"	"	36.4			
	"	"	35.5			
				36.8	1200	30.1
	2 v 25					
<i>B</i>	11 a.m.	15.5	—			
	"	"	37.5			
	"	"	37.0			
	4 v 25					
	7 p.m.	17.7	33.1			
	"	"	33.7			
	5 v 25					
	10 a.m.	17.9	32.9			
				33.6	1120	30.0
	"	"	33.5			
	2 p.m.	18.1	34.4			
	"	"	34.0			

When the plasma was diluted with Ringer a fall was encountered in the osmotic pressure greater than could be attributed to the proportional fall in protein concentration (see Table IV).

TABLE IV. 8 c.c. blood drawn from arm vein into 0.1 c.c. saturated potassium oxalate. Plasma diluted 1:1 with Ringer (RSB). Osmometers set up as usual and exposed to initial counter pressure of 16 cm. H₂O in triplicate at 4 p.m., 8 v 25.

	Time	Temp. ° C.	Osmotic pressure cm. H ₂ O	Mean value	Protein nitrogen mgm. p.c.	Osmotic pressure per gram. protein nitrogen p.c.
<i>A</i>	8 v 25					
	10 a.m.	19.8	12.8			
	"	"	11.1			
	"	"	10.7			
	3 15 p.m.	20.0	13.0			
	"	"	11.0	11.5	550	20.9
	"	"	10.6			

It was of interest to investigate this further and consequently a series of experiments was carried out, the oxalated plasma of the same subject being diluted to varying degrees with Ringer both with and without bicarbonate, and the resultant protein osmotic pressures determined in the manner already described. The results are summarised in Table V

TABLE V

Temp °C	Protein nitrogen mgrm p c. =PN	Osmotic pressure cm H ₂ O =p	Osmotic pressure per grm. PN p c.	1/p	ν ie 1/PN	Nature of diluent	Nature of dialysate
19 0	1504	49 8	33 2	67 5	74 5	—	RSB
17 9	1120	33 6	30 0	100	100	—	RSB
25 5	1047	32 4	29 6	103 5	107	—	RSB
16 3	1025	29 5	28 7	114	109	—	RSB
18 8	1010	28 2	28 0	119	111	—	RCB
25 3	1005	27 5	27 4	122	112	—	RSB
16 7	992	29 2	29 4	115	113	—	RSB
17 5	940	20 0	21 3	168	119	RCB	RCB
18 8	740	17 1	23 1	197	151	RCB	RCB
16 7	725	15 8	21 8	213	154	RSB	RSB
18 8	700	14 9	21 3	226	160	RCB	RCB
25 3	675	13 6	20 2	248	166	RCB	RSB
25 5	670	13 4	20 0	250	167	RSB	RSB
16 3	618	12 9	20 8	261	181	RSB	RSB
25 5	606	13 3	21 6	257	185	RCB	RSB
17 5	570	11 7	20 6	287	197	RCB	RCB
19 9	550	11 5	20 8	292	204	RSB	RSB
16 7	538	10 8	20 1	311	208	RSB	RSB
17 5	500	7 4	14 8	454	224	RCB	RCB
16 3	442	6 6	14 9	510	253	RSB	RSB

RCB=Ringer's fluid with 0.02 p c. NaHCO₃

RSB=Ringer's fluid without NaHCO₃

The figures in the first row are those obtained from plasma concentrated by ultra filtration. Those in the second row are taken as the normal plasma values, with which the remainder are compared in columns 5 and 6

It will be seen that the osmotic pressure per grm protein nitrogen p c gradually falls as the plasma is diluted. Adair⁽⁶⁾ has observed this phenomenon in the case of hæmoglobin dissolved in either *N*/10 NaCl, or⁽⁷⁾ distilled water. The possible cause for this which first occurred to the mind was that the molecular volume of the colloidal particles was comparatively large, and that one was therefore dealing with a solution in a state analogous to that exhibited by a gas when highly compressed. The reciprocal of the osmotic pressure and the reciprocal of the protein nitrogen were therefore calculated, the value of each for normal plasma being taken arbitrarily as 100, the values for the diluted and concentrated plasmas being interpreted in figures relative to this. The figures are given in columns 5 and 6 and are plotted in Fig 5. It will be seen that for dilutions ranging up to 50 p c of the original concentration, the points

he fairly accurately on a straight line¹ The figures in the first line of Table V were obtained from a plasma which had been concentrated by

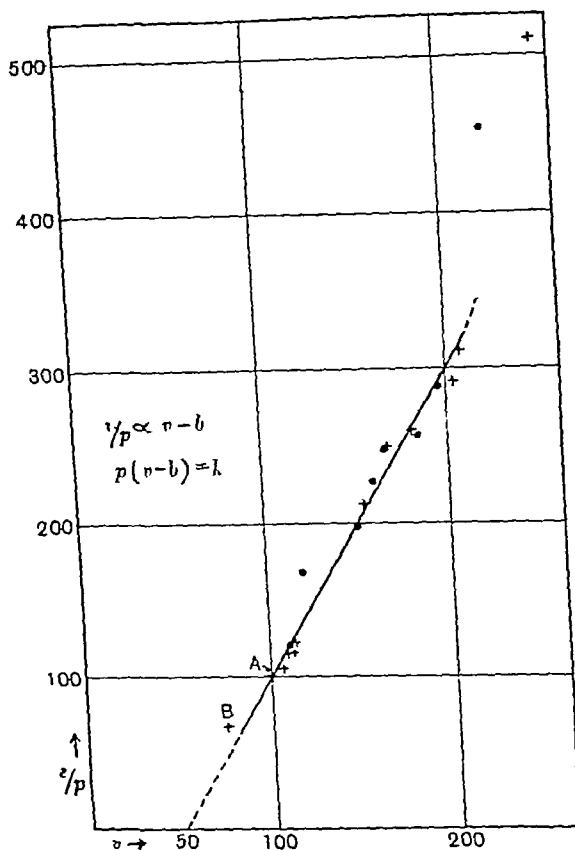


Fig 5 A, original plasma B, plasma concentrated by ultrafiltration.

$$\text{Ordinate} = \frac{\text{Osmotic Pressure Original Plasma}}{\text{Osmotic Pressure Diluted Plasma}} \times 100$$

$$\text{Abscissa} = \frac{\text{Protein Nitrogen Original Plasma}}{\text{Protein Nitrogen Diluted Plasma}} \times 100$$

+ represents readings when bicarbonate free Ringer, and ● readings when bicarbonate Ringer, was used as the diluent

ultrafiltration through cellophane, and it will be observed that the plotted point for this concentrated plasma lies in the neighbourhood of the same straight line It seemed to make no appreciable difference to

¹ Dr G S Adair informs me that he has obtained a similar form of curve in the case of sheep and of horse plasma with protein concentrations ranging from 1 p c. to 14 p c.

the values obtained whether the plasma were diluted with Ringer containing bicarbonate or no bicarbonate. Dilutions in the neighbourhood of 40 p.c. of the original concentration give rise to a relatively smaller pressure and fall distinctly above the line to which the figures of the more concentrated solutions adhere.

If this line be produced it will be seen that it cuts the v axis at the value of 50. In other words, the osmotic pressure of the plasma proteins reacts to their dilution, within limits, in a manner such as would be expected of a non-ionised colloidal solution in which the colloidal molecules occupied an effective volume as large as 50 p.c. of the original

SUMMARY AND CONCLUSIONS

1 The osmotic pressure of the proteins of human serum and plasma has been determined

2 Dilution of the plasma with Ringer's fluid gives rise to a relatively larger fall in osmotic pressure than the concomitant fall in the protein concentration

3 If p = the osmotic pressure of the proteins, v = the reciprocal of the protein nitrogen, and b = a constant, the relation $p(v - b) = k$ is shown to hold for dilutions of the plasma up to 50 p.c. of the original concentration

4 The constant b has a value of 50 p.c. of the original volume of the plasma

The expenses of this research were defrayed in part out of a grant from the Government Grant Committee of the Royal Society

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THE BLOOD COUNT AND BODY TEMPERATURE IN NORMAL RATS BY ARTHUR DIGHTON STAMMERS

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Johannesburg)

In connection with the maintenance of a colony of rats for experimental purposes, I have made observations to determine how far the height at which they live above sea-level, viz 6000 feet, influences the physiological processes. I give here observations on blood count and on body temperature

I THE BLOOD COUNT

Red corpuscles The figures which are available for comparison are few in number and, as far as I have been able to ascertain, relate to counts made at approximately sea-level. It is, of course, well known that, in man, the number of red cells normally present in the blood bears a definite relation to the altitude at which the individual is living. In Johannesburg (6000 feet) it is customary to regard the normal red cell count in man as 6 millions as opposed to 5 millions at sea-level, i.e. with a diminished barometric pressure of 140 mm Hg there is an increase of 20 p.c. in the red corpuscles.

The references may be briefly cited as follows. Donaldson⁽¹⁾ quotes work by Rivas, in which the average red cell count in ten rats was 8.2 millions per c.mm. and by Margot, who found that adults aged 148 days had a count of 9.4 millions.

Scott⁽²⁾ states that, in his colony, the normal is 8.8 millions, while Bedson and Zilva⁽³⁾ found 7.8, and Cramer, Drew and Mottram⁽⁴⁾ between 9 and 10 millions. The average of all these works out at about 8.7 millions for adult animals. In these latter reports the number of cases examined is not stated.

I have made counts in 60 rats over varying periods from 1922-1925, the results are as follows

Cases observed	Average red cell count
60	9.2 millions

The lowest count observed was 7.4 and the highest 10.6 millions, the distribution is shown in the subjoined table

Red cells (millions)	No of cases	Red cells (millions)	No of cases
7 4	1	9 3	4
7 5	1	9 4	6
8 0	1	9 5	4
8 4	1	9 6	3
8 6	2	9 8	3
8 7	4	9 9	3
8 8	3	10 0	1
8 9	7	10 1	1
9 0	2	10 2	1
9 1	3	10 3	2
9 2	6	10 6	1

If it be assumed that an increase similar to that observed in man results from life at an altitude of 6000 feet, the average which might be expected would be in the region of 10 4 millions. There is, however, no *a priori* reason for this, since, as will be seen later, considerable differences exist between human and rat blood and normal conditions in the latter appear to permit far wider fluctuations in the cell content than in the case of human blood.

Leucocytes As regards the leucocyte count, the average numbers observed by different authors are summarised in the following table. These all refer to rats in the Wistar Institute colony, Philadelphia, during the period 1914-1921, in which Donaldson reports that the nutritional state was good.

Authors	No of cases and sex	Av no of leucocytes	Poly morphs	Small lymphocytes	Others
Rivas	10	9017	55 10 p c	38 20 p c	6 70 p c
Newrey	11 ♂	9281	62 07	29 55	8 38
"	12 ♀	8555	55 71	30 92	13 37
Margot	3 ♂	9375	59 00	33 80	7 10

In observations on the 60 cases mentioned above, I obtained the following figures. The animals were all young healthy adults, weighing between 160 and 250 gm and aged between 70 and 200 days.

No of cases and sex	Total leucocytes	Poly morphs	Small lymphocytes	Others
30 ♂	10200	41 0 p c.	52 5 p c	6 5 p c
30 ♀	9400	39 0	54 2	6 8

The differences between these and the figures obtained by the authors mentioned above are somewhat striking, particularly as regards the proportion of polymorphs to small lymphocytes. The explanation for this is somewhat obscure, since the nutritional state of the animals was good and they were apparently in perfect health. It has, however, been pointed out by Donaldson(5) that, in the experience of other observers (Kleineberger and Karl, Eyre, Cramer, Drew and

Mottram), the percentage values for small lymphocytes may be about twice those for the polymorphonuclear cells and it is concluded that the blood picture may vary within wide limits and yet the animals remain in good health

Blood platelets The only comparative figures which are available on the blood platelets are those compiled by Cramer, Drew and Mottram(4), who state that the normal rats in their colony have an average count of about 0.8 million, and by Bedson and Zilva(3), whose normal rats gave a figure of about 1 million

Since the platelets disintegrate very readily in shed blood, the number found in a blood count depends upon the completeness of preservation and, in order to guard as far as possible against incomplete preservation, I have compared the results obtained with several preservative fluids

I have found no difficulty in distinguishing the platelets from bacteria, lipid granules and other small particles and, since the platelets are about 3μ in diameter, neither great magnification nor high resolving power (as has been pointed out by Bedson and Zilva) is required to see them

Cattoretta(6) states that digestion causes a decrease in the number of platelets in circulating blood lasting $1\frac{1}{2}$ –2 hours, in order to avoid this possible source of variation, my counts in rats were invariably made about 3 hours after a meal

The technique adopted was a slight modification of that of Cramer, Drew and Mottram and was as follows: the rat was anaesthetised with ether and the tail immersed in warm water for a few moments and dried. It was then cut about 2 cm. from the end, whilst immersed in Toison's fluid. When the blood was flowing freely, the tail was transferred to another dish of Toison's fluid and the blood allowed to flow until a dilution suitable for counting was obtained. The tail was then removed, dried and blood taken in the ordinary way for a red cell count and for a differential film. The rat was then bled into 2 p.c. sodium citrate in normal saline and into 0.02 p.c. methyl violet saline in the same way as described for Toison's fluid.

The blood was mixed in each dish with a platinum loop, a drop placed on a slide and a cover slip applied which was ringed with paraffin and set aside while the red cell count was made. The differential film was stained with Leishman's fluid.

The proportion of red cells to platelets was estimated by means of an ocular micrometer ruled in squares and the red cell count was carried

out with the Standard American Hæmocytometer with the Levy counting chamber This was tested and found to give results concordant with those from the Thoma-Zeiss instrument used by Cramer, Drew and Mottram

Twenty-seven rats in all were examined in the way described and the results are summarised in the subjoined table Not less than 50 platelets were counted in each case

	Red cell count	Diff film	Toison's fluid	Citrate saline	Meth. violet saline
Maximum variation	10 3	0 974	1 120	0 998	1 089
Minimum variation	8 9	0 870	0 877	0 869	0 854
Average of total	9 4	0 861	0 892	0 887	0 894
Red cell platelet ratio	—	10 9	10 5	10 6	10 5

The average variations are insignificant and well within the limits of experimental error The differential films show a definitely lower count and this is, in my opinion, explained by the greater difficulty in counting the platelets by this method and to the fact that more uncertainty exists as to the identity of the true platelets

As a control, the number of platelets in human blood was also investigated The finger was pricked and a platinum loopful of the blood was placed in each of the fluids already mentioned The first six estimations gave the results tabulated below

	Red cell count	Toison's fluid	Citrate saline	Meth. violet saline
Average of 6	6 1	0 680	0 723	0 775

The differences observed suggested that delay was a factor in the production of the relatively high count in the case of the citrate and methyl violet saline fixatives It was accordingly decided to make further estimations with each diluting solution and for this purpose to take blood from different fingers, thus ensuring that approximately the same time should elapse between pricking the finger and immersing the loopful of blood in the diluent Ten further estimations gave the following figures

	Red cell count	Toison's fluid	Citrate saline	Meth. violet saline
Average of 10	5 9	0 662	0 675	0 670

It will be seen that a considerably closer approximation was obtained when the estimations were made in this manner

Since, in human blood, delay was found to increase, instead of, as might be expected, diminishing the number of platelets, the effect of

delay in fixing the blood was tried on the blood of rats. A loopful of blood was obtained from the tail of the rat at given intervals and placed in 1 c c of citrate saline, the subsequent technique being as previously described. Twelve different rats were investigated in this way with the following results

	At once	After 30 secs	After 60 secs
Average of 12	0.874	0.898	0.976

The figures obtained in this way seem to indicate definitely that a pronounced increase takes place in the platelet count as a result of delay in fixing the blood.

Particles similar in appearance to platelets have been described as being produced in great numbers in plasma on cooling it and some observers indeed have considered that, in mammals, platelets are not normally present in the circulation. Whilst the results I have just given do not show that platelets are absent normally, they indicate that particles indistinguishable from platelets are readily produced in blood.

II BODY TEMPERATURE

In a recent communication, Price-Jones(7) states that an investigation into the body temperature of 200 normal rats showed a mean of 100.6°F , the limits being 96.6 to 103° . A table is given in this author's paper, which shows the distribution of temperatures in intervals of 0.2° and a graph is also inserted, in which the numbers of animals are plotted against the temperature in 1° intervals. From these data, the standard deviation and the coefficient of variation are calculated, the figures given being $1.13 =$ standard deviation, and $1.10 =$ coefficient of variation.

In connection with other investigations, it became necessary to determine the body temperature of my rats and the figures obtained are now presented. The average of 83 cases was 100.4° , the range being from 96.8 to 102.2° . The distribution was as follows

Degrees F	No. of animals	Degrees F	No. of animals
96.8	1	100.4	12
97.4	1	100.6	9
98.8	1	100.8	8
99.0	1	101.0	5
99.2	3	101.2	7
99.4	4	101.4	2
99.8	8	101.6	6
100.0	6	101.8	2
100.2	6	102.2	1

The standard deviation and the coefficient of variation, which were kindly worked out for me by one of my colleagues, Dr I Liknaitzky,

both give the figure 0.88. For purposes of comparison with Price-Jones' results, a graph is appended, expressing the same relative information

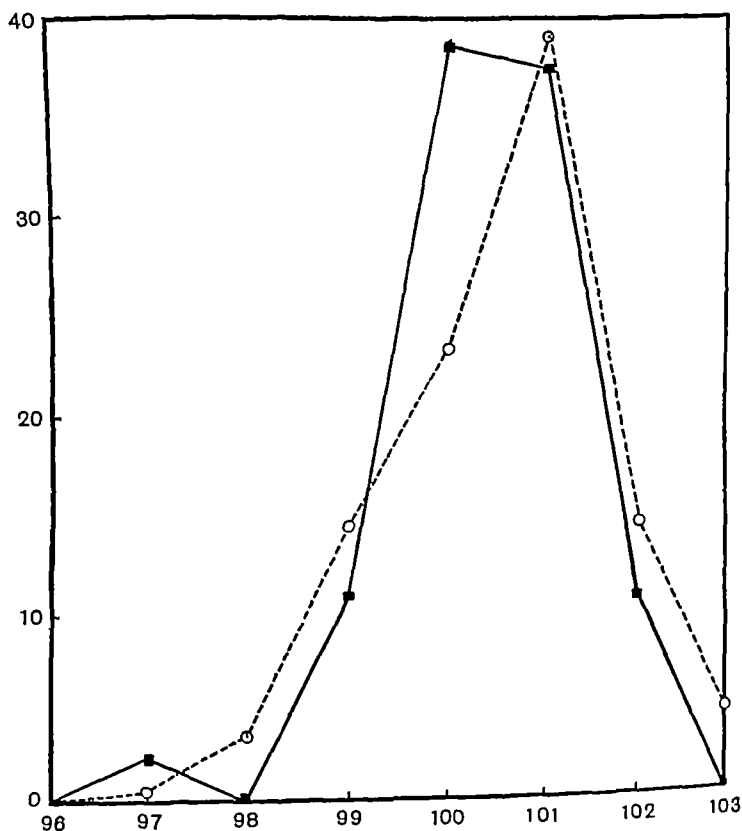


Fig 1 The ordinates indicate the number of animals, the abscissae the temperature groups (97 = 96½–97½, etc.) The dotted line refers to Price-Jones' animals and the continuous line to the writer's. Both have been reduced to percentages of the totals.

It may be mentioned that, as far as can be ascertained, the points in his graph do not agree with the figures in his table. These points occur as follows:

Degrees F	No. of animals
97	1
98	8
99	23
100	55
101	72
102	35
103	9

It is presumably intended that each full degree on the abscissa of the graph shall include temperatures half a degree below and above it, and in this case the figures would appear to be as follows

Degrees F	No of animals
97	1
98	7
99	29
100	47
101	78
102	29
103	9

The liberty has been taken of re-drawing Price-Jones' curve and including it in the graph illustrating the writer's results and, in order to make comparison possible, in view of the difference in numbers, both have been reduced to percentages of the totals

According to Pembrey(8), the average temperature of the adult albino rat is 99.5°, while Macleod(9) finds a mean of 100.2°. It will thus be seen that the figures obtained by the writer as well as by Price-Jones exceed those previously reported. This increase, however, is not regarded as of any significance, since, as the latter author points out, the temperature of a rat may fluctuate within 2 or 3° F under apparently the same conditions

The following figures bear out this statement. They were obtained by taking the temperatures on three successive days at as nearly as possible the same time

1st day	2nd day	3rd day
Max. temperature	Max. variation	Max. variation
101.8°	1.4°	3.0°
	(100.8 - 99.4)	(100.4 - 97.4)
Min. temperature	Min. variation	Min. variation
98.8°	0.2°	0.2°
	No variation. Two cases	No variation. Four cases
One animal showed a variation of only 0.2° throughout the three days		

In each case the average for the day works out at 100.4°. The maximum range for any one animal in 48 hours is 4° and the minimum 0.2°, the average being 1.1°. The room temperature was 50.9° on the first day and 51.8° on the others. This investigation was made during June, which is usually the coldest month of the year in Johannesburg, the mean minimum temperature being recorded as 41° F

Other observers, according to Donaldson, have found that an increase in body temperature occurs with an increase in room temperature up to 86°, the rate being 1.26° for the body with 9° increase in room

both give the figure 0.88. For purposes of comparison with Price Jones' results, a graph is appended, expressing the same relative information

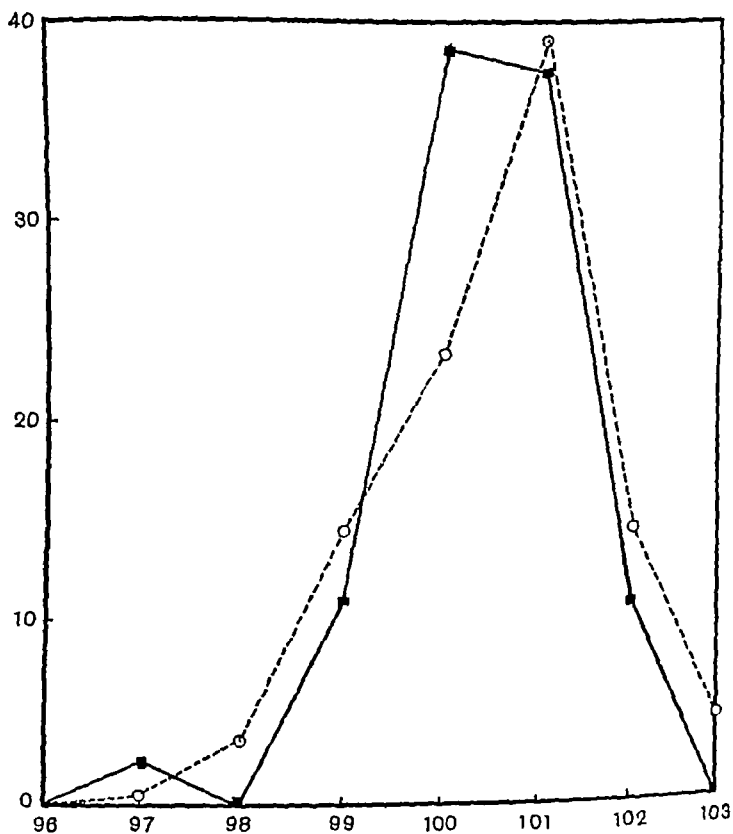


Fig. 1. The ordinates indicate the number of animals, the abscissae the temperature groups (97 = $96\frac{1}{2}$ – $97\frac{1}{2}$ etc.). The dotted line refers to Price Jones' animals and the continuous line to the writer's. Both have been reduced to percentages of the totals.

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Other observers, according to Donaldson, have found that an increase in body temperature occurs with an increase in room temperature up to 86°, the rate being 1.26° for the body with 9° increase in room

temperature The temperature regulating mechanism in the rat will thus be seen to lack efficiency

SUMMARY

The normal red cell count in rats appears, from what few investigations have been made, to be in the region of about 8.7 millions per c mm at sea-level. The author's observations at an altitude of 6000 feet give an average count of 9.2 millions, which represents an increase of under 6 p c as compared with the 20 p c increase seen in normal human blood at this altitude.

In the same animals the average leucocyte count was 9800, while the average reported by other workers is 9057. The proportion of polymorphonuclear leucocytes to small lymphocytes averaged 40-53.35.

The blood platelet count gave an average of just under 900,000. Comparisons were made with different diluents, since there appear to be complicating factors which influence the correct estimation of the platelets. No significant differences were observed with the three diluting solutions employed and a lower count obtained with a differential film is attributed to a greater difficulty in counting by this method. Delay in fixing the blood appears to be an important factor in determining the number of platelets.

The range of body temperature of the rats in the writer's colony was 96.8-102.2°. The standard deviation and the coefficient of variation were both 0.88. A graph illustrating the distribution of temperature as compared with that found by Price-Jones is appended.

It seems that, under normal circumstances and apparently the same conditions, the temperature of the rat may vary within 2 or 3° F, and this animal may be regarded as being somewhat poikilothermous.

The author desires to acknowledge assistance from the Research Grant Board of the Union of South Africa towards the expenses incurred in carrying out this work.

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STUDIES ON THE RELATION OF WORK AND HEAT
IN TORTOISE MUSCLE BY JEFFRIES WYMAN, JR
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(From the Department of Physiology and Biochemistry,
University College, London)

I *The Problem* Several observers recently have studied the relation of tension and work to shortening or stretching, at various speeds, in the case of voluntary muscle. A. V. Hill⁽¹⁾ and Lupton⁽²⁾ have shown that the work obtainable from human arm muscles accelerating an inertia wheel varies with the moment of inertia of the wheel, that is, with the time occupied by the shortening of the muscles. The results of their experiments could be quantitatively explained by assuming a certain maximum value for the work, corresponding to infinitely slow shortening, and by supposing that the difference between this value and that obtained in any actual shortening of finite duration was proportional to the speed of shortening. Gasser and Hill⁽³⁾ have found that the sudden release of an isolated frog's muscle during stimulation is accompanied by a fall of tension below the isometric value for the new length. The fall is followed by a re-development. They also found that stretching a muscle may lead to a temporary rise of tension above the isometric value for the new length. These workers have all suggested that these effects are to be ascribed to the "viscous" properties of the muscle, which resists a change of shape. Thus, when a muscle contracts and shortens, a certain amount of its potential energy is wasted in overcoming the resistance of the muscle substance itself, and appears as heat in the muscle instead of as external work. If the same muscle be stretched, the work done is greater than that required to overcome the elastic forces of the muscle by an amount due to the viscous and frictional resistance to deformation.

Long ago Fick⁽⁴⁾ remarked the same phenomenon in isolated frog muscle. He showed that greater tensions existed in a tetanised muscle which was being stretched than in the same muscle when shortening. He however ascribed the discrepancy to a greater responsiveness in the muscle being stretched. He assumed that stretching, although itself

incapable of exciting a muscle, could nevertheless increase the response to a given stimulus

In the following paper certain aspects of the question, those relating to work, are re-opened, and an effort is made to provide evidence for a decision between the two views, that of Fick and that of the other observers mentioned above. The experiments carried out for this purpose serve also to suggest a view as to the relation between different forms of energy in the muscle system, which is discussed at the end of the paper.

II *Method—theoretical* It is of course obvious that there must be an element of truth in the view of Hill and his colleagues. The irreversible nature of all actual processes demands the appearance of a certain amount of heat in the shortening or stretching. The best of springs is not wholly free from viscosity and hysteresis. A muscle, different as it is from a spring, and possessed of a complex colloidal structure, suggests the possibility of very considerable irreversible effects. The question, therefore, is whether the view of Fick is to be retained in addition to that of A. V. Hill, whether the supposed physiological consequence of a stretch is to be invoked in addition to its undeniable physical effect, in order to explain the results. Our chief interest lies therefore in finding and measuring some property of stimulated muscle which may be taken as a measure of its physiological response to the stimulus. We can then measure this property of a muscle while it is (a) at rest, or (b) in process of shortening, or (c) being stretched, and compare the results of the measurements in the several cases. If they are the same, or if the result is greater in the case of shortening than in the case of stretching, we conclude that Fick's interpretation is disproved, otherwise it is substantiated.

One such quantity is the heat liberated by the muscle. This heat is a measure of the magnitude of the chemical changes set off in the muscle by the stimulus, and the extent of these chemical changes may reasonably be taken as what we mean by the size of the physiological response. In order therefore to test Fick's hypothesis on the basis of this criterion we have alternately to stretch and release a tetanised muscle and to compare the heats produced in the two cases. These heats, however, cannot be taken as they stand. In the case of a stretch work is done on the muscle by the external, stretching force, in the case of a release work is done by the shortening muscle on the external system to which it is attached. These amounts of work must affect the measured heat. Consider the case of a stretch where the muscle is drawn out from length (2) to length (1), work ${}_2W_1$ being done upon it. Some of this work,

namely, that done against the frictional and viscous resistance of the muscle to change of shape, will appear directly as heat. The remaining part will be used in increasing the mechanical potential energy of the muscle *qua* elastic body. This mechanical potential energy, however, may be assumed to be converted into heat at the moment of its disappearance in relaxation (an assumption to be discussed in detail at the end of the paper). In order, therefore, to obtain the true heat produced by the muscle itself during the operation we must subtract from the measured heat the work ${}_2W_1$. Similar considerations apply to the case of a release. Here the muscle shortens with a loss of potential energy, so that less heat is liberated in relaxation than would have appeared at the greater length (according to the assumption just mentioned). Part of this lost potential energy is used in overcoming the resistance of the muscle to change of shape, as in the case of a stretch, and appears directly as heat. The remaining part appears as the external work ${}_1W_2$ done by the muscle in shortening, and is lost to the muscle system. Accordingly, to obtain the corrected heat we add to the measured heat an amount of work ${}_1W_2$.

III *Method—technical* The muscle employed for these experiments was the *biceps cruris* of the tortoise. This is a long, uniform muscle running from the pelvis to the ankle. It serves both for locomotion and to hold the hind leg drawn in when the animal is frightened. It is chiefly useful in such an investigation as this because of its slowness of action. It may be tetanised for about 10 seconds without showing signs of fatigue. This capacity to maintain tension for such long intervals, even in the isolated condition, is to be explained no doubt in part by the extreme general slowness of the tortoise, and in part by the function of the muscle in maintaining the legs in a flexed position for long periods of time when the animal is frightened and in danger. The muscle is also useful in showing no harmful effects from even very quick stretches (2-3 cm per sec). In this respect it stands in marked contrast to the muscles of the frog, with which indeed it would have been impossible to carry out these experiments at all.

The measurements of heat are made according to the general methods developed by A. V. Hill, which need not be here described. The thermopile employed is of the type shown in (5), Fig. 2, p. 239. The muscle is supported by the bone attachment at the pelvic end in a glass clamp fixed to the thermopile and bearing one electrode. The flat face of the muscle lies over one set of junctions of the thermopile. Its free end is secured to a wire rod bearing the top electrode and connecting it with

the device for measuring work. The electrodes are used both for the stimulating and calibrating currents, thus the calibrating current passes through the full length of the muscle. The whole rigid structure bearing muscle and thermopile is contained in a moist chamber filled with oxygen and kept at very constant temperature inside the usual Dewar flask. The wire connecting the free end of the muscle with the device for measuring work passes out of the moist chamber through a long, narrow glass tube, as in previous cases where heat and tension have been measured together. In this way simultaneous measurements of heat and work can be made.

The device referred to for measuring work produces a continuous tension-length curve for the muscle throughout a stretch or release. It is analogous, but not similar in design, to the Blx Myograph figured in Fick's book (4), p. 23. It gives, in curvilinear coordinates, an "indicator

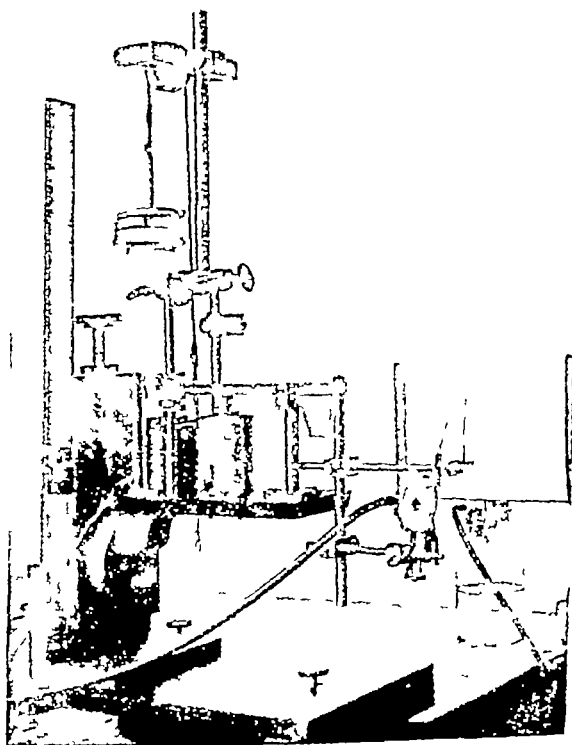


Fig. 1 Photograph of device used for writing continuous tension-length curves of muscle during shortening or stretching

diagram" for the working muscle. In this diagram ordinates give displacements of the free end of the muscle, abscissæ tensions, and consequently the area of the diagram is a measure of the work done on or by the muscle. It is best explained with reference to the accompanying diagram (Fig 1 *a*). The photograph (Fig 1) will also serve to make the

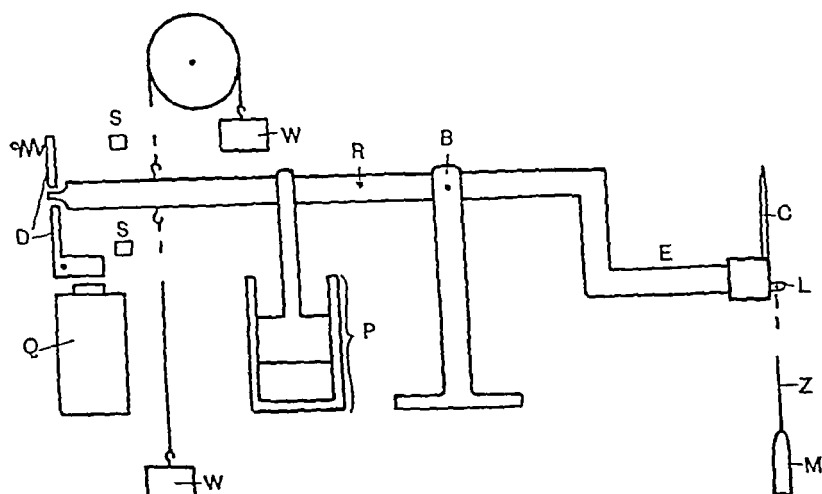


Fig 1 *a* Diagram of device shown in Fig 1

matter clearer. *L* is an isometric lever to which the free end of the muscle *M* is attached by the wire *Z*. The lever *L* is rigidly fixed, by an L-shaped metal connection *E*, to a brass rod *R*, which may be rotated about an axis *B*, perpendicular to the plane of the diagram, by suitably suspended weights *W*. The angle of rotation is controlled by adjustable stops *S*. *R* is held in an initial horizontal position, until the moment of release, by a catch *D* controlled by an electromagnet *Q*. The speed of rotation is constant, due to nearly instantaneous critical damping by the dash-pot *P*, it may be adjusted at will by the weights employed, and by a tap belonging to the dash-pot. The lever *L* is equipped with a writing pointer *C*, which is perpendicular to *R* (in the plane of the figure) when the lever is not under tension. The tip of *C* lies in the line of *R* (produced). Thus, when *R* is rotated from its resting horizontal position through a small angle, the tip of *C* describes what is very nearly a straight vertical line, provided the lever is not under tension. If, on the other hand, the tip of *C* is displaced from the position of zero tension, *R* remaining stationary, it will describe an arc of a circle of radius equal to the length of *C*, cutting this vertical line at right angles. When the

stimulated muscle under tension is stretched, or is allowed to shorten, the tip of C will thus receive both horizontal and vertical displacements, and will describe a curve like that shown in Fig 2 Actual curves, traced by the pointer on smoked paper, are given in Fig 3

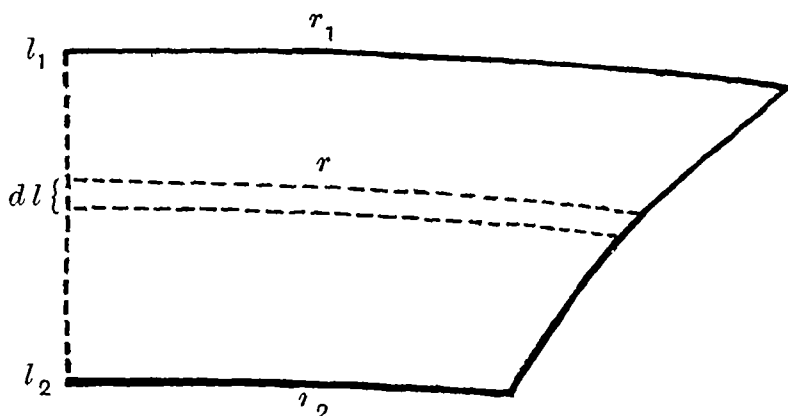


Fig 2 Diagram of a tension length curve horizontally tension, vertically length of muscle

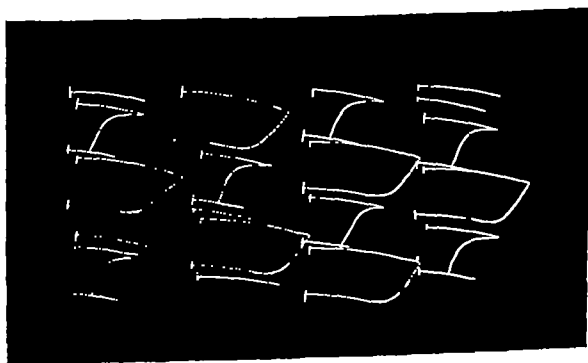


Fig 3 Tension length curves recorded for *biceps cruris* Small curves are for releases large curves for stretches Isolated lines represent isometric contractions in the 'long' or 'short' positions.

That the curves really serve to measure the work may be shown as follows. Since the arcs of horizontal displacement are small, not differing sensibly from the chords for cases covered by the experiments, and since they all cut the vertical line of zero tension at right angles, they may be taken as parallel without appreciable error, and the area of the curve, closed by the line of zero tension, may be taken as nearly equal to $\int_{l_2}^{l_1} r dl$ Here dl (see Fig 2) denotes an element of vertical displacement at any length l , and r the corresponding horizontal displacement But dl can be shown as follows to be equal to the vertical displacement

of the free end of the muscle. Let B , C and L in Fig. 4 refer respectively to the axis of rotation of the bar, the tip of the pointer in the position of zero tension, and the long axis

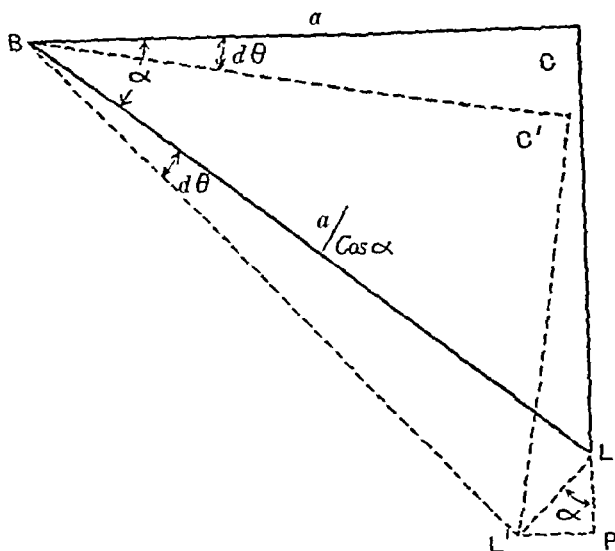


Fig. 4. Diagram of small displacement of lever

of the spring of the lever. Let $BC = a$, and $\angle CBL = \alpha$. The triangle CBL is rigid, and moves as a whole (e.g. to $C'BL'$) when the bar R is rotated. Suppose now that R is rotated through an angle $d\theta$. Then C traces a vertical element $dl = a d\theta$, and at the same time L traces an element $LL' = \frac{a d\theta}{\cos \alpha}$. This element makes an angle α with the vertical, and has a vertical component $LP = LL' \cos \alpha = a d\theta = dl$. The free end of the muscle being attached to L , is thus given a vertical displacement dl . Furthermore, returning to Fig. 2, r is proportional to the tension exerted by the muscle, the spring being so chosen that over the range of forces exerted by the muscle $f = Kr$ where f denotes force and K is a constant. Consequently

$$\int_{l_2}^{l_1} r dl = \int_{l_2}^{l_1} \frac{f}{K} dl = \frac{1}{K} \int_{l_2}^{l_1} f dl,$$

where $\int_{l_2}^{l_1} f dl$ is the work done by, or on, the muscle when its length changes from l_2 to l_1 .

By calibrating the spring we may determine K . Now we have seen that $\int_{l_2}^{l_1} r dl$ is sensibly equal to the area of the curve written by the tip of C completed by the line of zero tension. Consequently by measuring this area with a planimeter and multiplying by K we obtain directly the work done by or on, the muscle. It may be remarked that since the length of the wire joining the muscle to the lever is great the horizontal displacement of L will give rise to only a very slight horizontal displacement of the free end of the muscle.

In practice a light isotonic lever, as used by A. V. Hill (5), Fig. 3, p. 240) in recent measurements of heat production, was interposed between the muscle and the isometric lever, being fixed to the bar R . This was to ensure that the heat was measured (by the maximum

deflection of the galvanometer after the moment of relaxation) at the greater length of the muscle, the length at which the calibration was later carried out. Since this lever performed a complete cycle between the beginning of the stimulus and the maximum deflection of the galvanometer it introduced no additional corrections.

In an actual experiment the muscle is set up on the thermopile, as previously described, and attached to the lever *L* by the wire *Z*. It is adjusted so that its stretched length is just greater than its resting unloaded length. When this is the case the isometric heat at the greater length is nearly equal to that at the shorter length (about 7 mm. less). The existence of this approximate equality is essential to the argument. When the system has attained thermal equilibrium the point of the writing arm *C* is brought into contact with a plane surface of smoked paper on which it writes freely. At a given moment a tetanic stimulus is applied by the breaking of a short-circuit by one of the arms of a Lucas revolving contact breaker. After a fixed interval of time sufficient to allow development of maximum tension the circuit containing the electromagnet *Q* is broken by a second arm of the contact breaker, and the muscle is allowed to shorten, or is stretched, under tension and at a constant speed determined by the weights and the adjustment of the dash-pot. After the lapse of an interval great enough to allow completion of the movement the stimulus is removed by the breaking of the primary circuit of the induction coil by the third arm of the contact breaker. The reading of the galvanometer is then taken, the properties of thermopile and galvanometer being so chosen as to ensure that maximum deflection occurs only after relaxation is complete, without danger of heat having been lost by the thermopile in the interval¹.

In the meantime the pointer *C* has written the curve from which the work done by, or on, the muscle can be later calculated. Stretches and releases, as well as isometric "long" and "short" heat measurements, are made in this way in a definite order of rotation, each experiment having at least three cycles. It is of course essential to insure that the stretches and releases should always take place between the same lengths of the muscle. To allow of this the whole instrument for measuring work is mounted on a stand which can be raised and lowered by a screw adjustment. At the conclusion of the experiment the muscle

¹ The fulfilment of this condition may be tested at the end of the experiment by heating the dead muscle for various times, up to that occupied by the actual stretches and releases, by a constant alternating current. In this case the deflection of the galvanometer should be proportional to the duration of the heating up to the limit considered.

is calibrated in the usual way to allow for conversion of galvanometer readings into ergs

An objection may be raised, based on the contention that the system of the wire and lever (*Z* and *L*) is not truly isometric, so that the curves written by the pointer do not give the works actually done by or on the muscle. Beyond doubt the lever wire system is extensible, indeed, it may be shown by carrying out a complete cycle with it that it behaves like a very stiff spring possessed of negligible hysteresis. But precisely for this reason it follows that the errors in the measurements of work cancel one another, though the areas of the curves taken alone require corrections. Consider the case of a shortening muscle. Before release the muscle develops tension "isometrically" and does work not recorded on the diagram, since the pointer simply moves to the right. During the shortening the tension falls and the pointer moves back towards the left. At the same time the lever wire system, relieved of a certain amount of tension, must shorten. For this reason the vertical displacement on the tension length curve is greater than the true shortening of the muscle, and the work recorded is correspondingly too large. On relaxation the lever wire system shortens still further, actually doing work on the muscle not recorded at all. But since the lever wire system possesses no appreciable hysteresis, the excess work registered on the diagram and the work done on the muscle at relaxation together exactly nullify the unrecorded work done by the muscle "isometrically" before release. Similar considerations apply to the case of a stretch. The objection is thus seen to be groundless.

IV *Experimental results* The experiments carried out fall into two classes, those done in the spring of 1925 and those done in the following autumn. During the intervening summer the tortoises obtainable were found to be in bad condition and unsuitable for the experiments. The two sets of observations were alike in showing that the heats, measured and corrected according to the procedure described above, were unequal, the heats for the releases being clearly greater than those for the stretches. The size of the difference varied from experiment to experiment and was of the order of 30-40 p.c. for the mean values. The "isometric long" and the "isometric short" heats lay in general between the two. The data of a single typical experiment are given in Table I.

TABLE I. Results of Exp. 5, Set II.

Operation	Gross heat (10 ³ ergs)	Work (10 ³ ergs)	Net heat (10 ³ ergs)
Shortening	107	37	144
Stretch	204	118	86
Isometric long	103	—	103
short	123	—	123
Shortening	101	40	141
Stretch	222	120	102
Isometric long	110	—	110
short	129	—	129
Shortening	107	36	143
Stretch	221	113	108
Isometric long	109	—	109
short	129	—	129
Shortening	115	39	157
Stretch	224	116	108

It was thought desirable, since the muscles varied considerably according to their size and condition in the amounts of heat which they produced, to "reduce" the results so as to make them more strictly comparable. For this purpose the heats for stretch, release, "isometric long" and "isometric short" were averaged for each experiment, and the averages were then multiplied by a scale factor so chosen as to reduce the average value of the "isometric long" heat to 100,000 ergs. The results of the two sets of experiments, so treated, are given in Table II. It is easily seen that the two sets of experiments are very similar, though there is considerable variation among the individual experiments comprising each set. In order to get representative values of the different heats for the two sets, in view of the fact that the experiments of each set appeared, in the judgment of the observer, to be of various degrees of reliability, weights were assigned to each experiment, and weighted

TABLE II Summary of experiments

Exp	Weight	SET I.					
		Work (10^3 ergs)		Heat, corrected (10^3 ergs)			
		Stretch	Shortening	Isometric long	Isometric short	Stretch	Shortening
1	2	68	24	84	86	73	108
2	2	98	56	254	256	254	289
3	1	179	95	374	409	383	465
4	2	174	67	190	220	174	263
5	1	68	29	200	226	209	234
Same, reduced to 100,000 ergs for isometric long							
1	2	81	29	100	103	87	124
2	2	39	22	100	101	100	114
3	1	48	25	100	109	102	124
4	2	92	35	100	116	92	139
5	1	34	15	100	113	104	117
Weighted mean		63	27	100	108	95	124
		SET II.					
		Stretch	Shortening	Isometric long	Isometric short	Stretch	Shortening
		Stretch	Shortening	Isometric long	Isometric short	Stretch	Shortening
1	2	206	62	464	435	462	495
2	5	169	78	275	311	291	383
3	5	210	118	404	441	446	525
4	3	178	87	389	389	378	470
5	5	117	38	107	124	101	146
6	5	166	62	195	225	174	281
7	5	114	47	105	110	80	153
Same, reduced to 100,000 ergs for isometric long							
1	2	44	13	100	94	98	107
2	5	61	38	100	113	106	139
3	5	47	27	100	109	111	130
4	3	46	22	100	100	97	121
5	5	109	36	100	116	94	136
6	5	61	46	100	115	89	144
7	5	109	45	100	105	76	146
Weighted mean		72	36	100	107	96	135

means for the different heats for each set were obtained. These, together with the weights, are also given in Table II.

V *Discussion* It is at once evident that just the 'reverse of what would be expected from Fick's hypothesis is in fact the case: the test appears to be fatal to the hypothesis. To be sure it is observed that the "isometric long" and "isometric short" heats are not quite the same: the mean values for the "isometric short" heats are 7 p.c. or 8 p.c. greater than those for the "isometric long" heats. Thus, however, cannot possibly explain the much larger differences between the heats of "stretch" and "shortening", so we are left with the conclusion that the physiological response, as measured by heat production, is less for a stretch than for a shortening, whereas the work, recorded by the diagram, is greater. Consequently, the difference between the works (a) for a stretch and (b) for a release cannot be ascribed to a difference in physiological response of the muscle in the two cases, and we are constrained to fall back on the explanation offered by Hill and his co-workers, unless at least some third alternative is offered.

So much as regards work. But how are we to account for the phenomena in the case of the heats? We have just seen that the heats of shortening are always considerably greater than those of stretching. Are we to make a new assumption just contrary to Fick's assumption? We might indeed suppose that there is a regulatory mechanism in the muscle which cuts down its response when it is being stretched and work is being done on it and which increases its response when it is actively shortening and itself doing work. Such a supposition, however, while giving an *ad hoc* explanation of the discrepancy of the heats, is not favoured by the results in regard to the works. It is moreover unnecessary: it is easy to show that without making any such hypothesis it is possible to explain the inequality of the heats by a simple modification of our initial assumption that the mechanical potential energy appears quantitatively as heat in relaxation.

Let us suppose that when the muscle relaxes only a fraction of its mechanical potential energy is transformed into heat: the rest appearing in some other guise e.g. as chemical energy (see A. V. Hill (5), Garner (6)). Then it is clear that if we stretch a muscle a fraction only of the work done on it in increasing its potential energy will appear as heat in relaxation. Consequently, in applying our corrections to the heats for the stretches we are subtracting too much from the observed values. Similarly, in the case of the releases we are adding too much, and we

should expect what in fact we find, that the corrected heat in the stretches is less than the corrected heat in the releases, even though the physiological response of the muscle is the same for both cases. It cannot be denied that by modifying our fundamental assumption in this way we weaken our case against Fick—we open a possible loophole of escape. Yet the matter is not serious. If we do not make this modification we are forced not only to reject Fick's view, but to accept the direct opposite. By introducing it we are enabled to explain the results without supposing that the physiological response of the muscle differs for stretches and releases, and we might conceivably go further still and insist on Fick's hypothesis. Yet such procedure not only appears highly gratuitous, but would demand, as will appear presently, an unexpectedly small value for the fraction of potential energy passing into heat at relaxation.

It is instructive to formulate the matter algebraically. For this purpose some discussion is necessary. If we stimulate a muscle isometrically for different times and plot total heat production (which here needs no correction) as ordinate against the corresponding duration of stimulus as abscissa, we get a curve which is approximately a straight

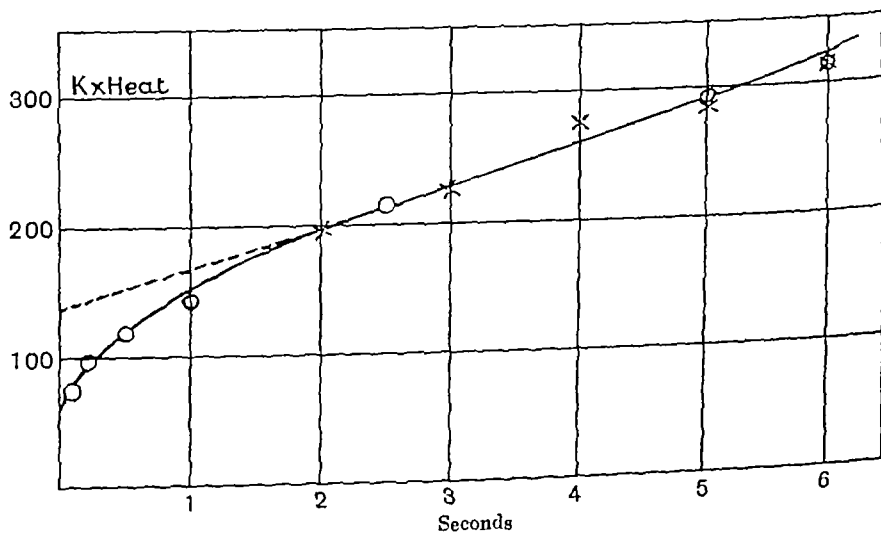


Fig 5 Curve showing relation of total heat produced (isometrically) to duration of stimulus for *biceps cruris* at about 12° C. O = muscle A, x = muscle B

line of positive slope. Such an experimental curve for *biceps cruris* is given in Fig 5. The equation of this line may be written $Q = A + Bt$

Here Q is to be interpreted as the total heat produced by the muscle A as the heat corresponding to a very short stimulus (just sufficient fully to excite the muscle), and B as the excess heat produced per unit time for a stimulus of finite duration. B is also equal to the slope of the line. Now Hill and Hartree's analyses have shown that the heat produced in a single twitch may be divided into two parts, of which one is the initial burst of heat and the other the heat given out on relaxation. We will therefore regard A as the sum of two terms like the above, and we will further identify the heat of relaxation with the disappearance of the mechanical potential energy of the contracting muscle. Thus, letting H denote the initial burst of heat, P the potential energy of the contracting muscle, and α the fraction of this energy appearing as heat at the moment of relaxation, we can write for the heat produced isometrically during a tetanus at any length l ,

$$Q = H - \alpha P - Bt$$

Over the range of lengths used in these experiments, B (giving the slope of the curve discussed above) may be taken as independent of the length of the muscle. Its effect is in any case not predominant. Consequently if l_1 is the greater, l_2 the shorter length of the muscle we may write for the isometric long and isometric short heats respectively

$$Q_1 = H_1 - \alpha P_1 - Bt$$

and

$$Q_2 = H_2 - \alpha P_2 - Bt$$

For the work of a stretch we write ${}_2W_1$ and for the work of a shortening ${}_1W_2$. These works, as recorded by our indicator diagrams, are not equal. ${}_2W_1$ is greater, ${}_1W_2$ less, than the thermodynamic work $P_1 - P_2$. In making our corrections it is the quantities ${}_1W_2$ and ${}_2W_1$ that we actually employ. Nevertheless since the differences between the actual works and the thermodynamic work appear either in our galvanometer readings or in our work curves and since in all our experiments the true isometric value of the tension for the final length (whether after a stretch or shortening) is always attained before relaxation, reflection will show that the effect of the corrections may be expressed in terms of the P 's. The corrected heat for a stretch then becomes

$$Q_{\text{stretch}} = H_2 - \alpha P_1 - Bt - (P_1 - P_2)$$

and that for a shortening

$$Q_{\text{shortening}} = H_1 - \alpha P_2 - Bt - (P_1 - P_2)$$

But since isometric long and isometric short heats are nearly equal,

i e $Q_1 \equiv Q_2$, we have $H_1 - H_2 \cong -\alpha (P_1 - P_2)$ Consequently the difference ($Q_{\text{shortening}} - Q_{\text{stretch}}$) is equal to

$$2(1 - \alpha)(P_1 - P_2)$$

This quantity is positive since α is by hypothesis a fraction, and P_1 is necessarily greater than P_2 (as has been found by the experiments described above) This simple hypothesis, therefore, regarding the fate of the mechanical potential energy of the muscle in relaxation serves quite as well to explain our results as any assumption of a "governor mechanism" in the muscle

If we know $(P_1 - P_2)$, that is, the thermodynamic work for change of length of the muscle from l_1 to l_2 , it is obviously possible to calculate $(1 - \alpha)$, *i e* the fraction of the mechanical energy of the excited muscle available in relaxation for chemical synthesis, etc But, since the stretches and releases were made at nearly the same speed, we shall not incur a very large error if we take $(P_1 - P_2)$ as the mean of ${}_1W_2$ and ${}_2W_1$ Making use of these values for $(P_1 - P_2)$ we get for $(1 - \alpha)$ the results shown in Table III

TABLE III. Values of $(1 - \alpha)$ for the various experiments.

SET I		SET II	
No of experiment	$(1 - \alpha)$	No of experiment	$(1 - \alpha)$
1	34	1	16
2	23	2	33
3	30	3	24
4	27	4	35
5	26	5	29
Weighted mean = 32 (weights as in Table II)		6	51
		7	49
		Weighted mean = 36 (weights as in Table II)	

It is worth noting that such a reabsorption of part of the muscular potential energy during relaxation would be of very great value to the animal This would be particularly the case for animals like the frog, whose locomotion is effected by twitches of the leg muscles rather than by any considerable shortening Indeed very seldom can muscles at work in the animal be supposed to shorten to the point where the isometric tension falls to zero, there must accordingly be a very considerable waste when, on relaxation, the potential energy of the contracted muscle passes over into heat Any provision for the utilisation of even part of this energy would be valuable

It is interesting to compare the magnitude of such a restoration—assuming its existence—with that due to aerobic recovery as estimated by

Hill and Meyerhof The total mechanical potential energy of a tortoise muscle at the resting unloaded length, reckoned from the work diagrams obtained in these experiments, is approximately equal (within about 10 p c) to the isometric heat for this length. This is in accord with previous observations on the relation between anaerobic heat and theoretical work. Thus, at the resting length of the muscle the amount of mechanical potential energy available for re-synthesis of breakdown products during relaxation is about 33 p c of the anaerobic heat production. Now Hill and Meyerhof(7) have shown that in aerobic recovery, of every 1 grm of lactic acid set free in the initial phases of contraction, about 0.8 grm is restored to its initial condition, the remainder being oxidised, so that the energy absorbed in aerobic recovery is about 80 p c of that given out in the preceding anaerobic breakdown. Consequently, the effect of the restoration discussed above would be, at the resting length of the muscle, between one-half and one-third of that described by A. V. Hill and Meyerhof, between one-third and one-quarter of the whole.

According to the above calculations about 36 p c of the potential energy of the contracting muscle is restored as chemical energy during relaxation. It is perhaps pertinent to note that of the energy liberated by oxidation in recovery about $\frac{80}{80+150}$, i.e. 35 p c, is stored as chemical energy in the re-synthesis of lactate into glycogen.

SUMMARY

1 A maximally tetanised skeletal muscle exerts a greater force while being stretched, a smaller force while shortening, than during an isometric contraction. This phenomenon has been attributed by A. V. Hill and his co-workers to irreversible physical factors such as viscosity, by Fick, however, who was familiar with the same fact, it was ascribed to a change in the 'physiological response of the muscle' evoked by the actual process of stretch or release. Experiments have been undertaken and are here described to decide between these alternative views. These experiments employ as the criterion of the "physiological response" the total energy liberated by the muscle.

2 When the *biceps cruris* muscle of the tortoise, undergoing a 4-6 seconds tetanus, is allowed to shorten, or is stretched, between two lengths the work done on it during the stretching is considerably greater than that done by it when it shortens, to an extent depending on the speed of movement. The corresponding total energies, on the other hand (heat - work and heat + work respectively), bear just the opposite relation, the energy liberated during a stretch being 30-40 p c less than

that liberated during a release Fick's hypothesis is consequently discredited the greater work is not associated with a greater liberation of energy—rather in fact the reverse We are left with the necessity of finding a physical explanation of the phenomenon

3 In carrying out the test described above it was assumed that the potential energy undeniably existing in the contracting muscle is degraded quantitatively into heat during relaxation This assumption is not necessary if we suppose that about one-third of this energy is reabsorbed during relaxation in helping to effect those chemical re-syntheses which are completed in oxidative recovery, then we are able quantitatively to explain the phenomena without having recourse to any hypothetical "governor mechanism" in the muscle

I have been indebted throughout the whole course of this research to the suggestions and encouragement of Prof A V Hill, whom I take this occasion to thank for his kindness The expenses of the work have been borne by a grant of the Royal Society to him

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THE CONDUCTION OF RESPIRATORY NERVOUS IMPULSES THROUGH REGIONS OF BLOCK PRODUCED BY PRESSURE

By GRACE BRISCOE, M B, B S

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School of Medicine for Women)

Introductory In almost any region of the body pathological changes may produce pressure on nerves of such intensity as to abolish the function of conduction. The phrenic nerve not uncommonly suffers in this way, and Charlton Briscoe(1) has shown that a definite syndrome of signs and symptoms is produced by the resulting unilateral paralysis of the diaphragm. His work, based on clinical cases and post-mortem evidence, suggests interesting problems connected with nerve-pressure block, the investigation of which has been the object of the following experiments.

He found various lesions causing pressure on the phrenic nerve and paralysis of the diaphragm, but though there commonly resulted a definite constriction or waist on the nerve trunk in no case could degenerative changes be demonstrated by the methods of staining of Marchi or Weigert Pal. Further, in cases of malignant disease the growth had frequently failed to infiltrate the fibrous sheath of the nerve and directly to implicate the conduction tissues. These observations suggest that simple mechanical constriction was the direct paralyzing agent, and not infiltration of the nerve proper or degeneration of its conducting elements.

The X-ray appearances suggested a second problem. Woodburn Morison(2) has shown that in "Eventratio Diaphragmatica" a congenital condition in which the muscular tissue on the affected side is undeveloped, elevation of this side is a constant phenomenon. In acquired paralysis of one side, however, he found that elevation might be slight, and in Briscoe's cases elevation was by no means constant, so that the question arises whether the diaphragm can retain tone after its nerve has lost the power of conducting motor impulses.

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lysed half of the diaphragm. Not only does it fail to descend, but it may ascend, while the sound half is contracting. This occurs during quiet respiration, but if a deep breath be taken these abnormal movements may be obscured. This may be explained as due to excessive action of the chest, or drag from the sound side, but the possibility remains that it might be due to the breaking through of the nerve block by stronger respiratory impulses.

The points which presented themselves for investigation were as follows. The amount of pressure necessary to cause loss of conduction of the nervous impulses which (a) subserve contraction, and (b) control tone, the possibility of stronger nervous impulses breaking through a region of block which is impenetrable to the impulses of normal quiet respiration.

Historical An account of the early work on Nerve Compression will be found in Luciani's *Physiology* (3). The point at issue was whether gradual compression would abolish conductivity in a nerve without any increased excitability. All the early experiments were carried out by crushing methods, the nerve being compressed by weights. Meak and Leaper, 1911 (4), set out to measure the pressure necessary to block conduction in a nerve, the pressure being transmitted by oil to the outside of a blood vessel, through which the nerve was passed. They found that the average time required to block the nerve impulse in the frog's sciatic, by means of a constant pressure of 40 lbs., was 7.5 minutes. Meak and Leaper do not state to what unit of area their pressure is applicable, but assuming that they mean 40 lbs. to the square inch, then this would be equivalent to a pressure of nearly $2\frac{1}{2}$ atmospheres, i.e. the height of a column of Hg 2026 mm. high.

Methods The main part of this investigation has been carried out on cats, using the diaphragm and the phrenics. A large number of experiments have also been made on gastrocnemius-sciatic preparations from the frog and toad. These have been regarded as supplementary to the phrenic experiments, and they will not be given in detail.

Thirty-three cats have been used. In the early experiments ether and urethane were employed as anæsthetics, and in the later ether and chloralose. Eight of the preparations were decerebrate, using Sherrington's trephine method.

The anæsthetised animal was firmly fastened to the table. The two domes of the diaphragm and the thorax were connected to the levers. The phrenic on one side of the neck was then dissected and finally pressure applied.

This method of recording the action of the diaphragm was chosen in preference to Head's slips, because the outline of the excursion of the domes is the movement seen during X-ray examination

To record the movements of the diaphragm a central incision was made in the abdominal wall. Hooks were fixed in the abdominal surfaces of the domes. Threads attached to these hooks emerged through small openings in the abdominal wall on each side of the central incision, and were connected to simple crank levers writing one above the other on a kymograph. It was found that the best way to prevent friction was to use vaseline freely on the threads and on the abdominal wounds. A short record was taken to see that the two levers were moving equally. Unless the two hooks are placed in approximately the same relative position there may be considerable difference in lever excursion on the two sides. Gunn's(5) rubber stethograph proved the most satisfactory method of recording chest movement. Inspiration is recorded as an upstroke in the chest movement, as a downstroke in the diaphragm movements.

When all the levers were writing satisfactorily a control tracing was taken. It is essential if changes of tone are to be recorded that there shall be no alteration of the levers or threads after the control tracing has been taken. The animal must be firmly secured to the table, and the dissection of the phrenic performed with everything in position, and the levers recording on the stationary drum. In this way any damage done to the nerve by dissection is demonstrated at once for the lever attached to the experimental side rises and its excursion is diminished. If this happens conduction may be restored by giving the nerve rest for a few minutes and placing a pad soaked in warm saline on the wound. If conduction does not completely recover the early changes of tone and movement are lost. Any struggle will of course vitiate the results. For this reason chloralose is the most useful anæsthetic and ether is only necessary for induction.

In using the word "tone" in this communication I am referring to the height of the diaphragm at the end of expiration. Sherrington(6) referring to the diaphragm as one of the muscles limiting the abdominal chamber, recalls that Dittler found that its action currents did not subside completely, even at the end of expiration, and suggests that since there is a residual degree of contraction still persisting, the diaphragm is in a state of "postural contraction" or "reflex tonus" at the end of the expiratory period. If, therefore, the expiratory level of the tracing drops on the experimental side this is regarded as a rise of tone, if the level rises, a loss of tone is argued. In this connection the tracing of the sound side acts as a control, as one leaflet of the diaphragm will maintain a uniform level and excursion over prolonged periods.

Care must be taken to identify all the roots of the phrenic. The nerve was always examined post-mortem, to see if all the roots had been compressed. There is such variation in the roots of the phrenic in the cat

that it is easy to miss one. Frequently the roots do not join together to form a common trunk until the nerve has passed into the thorax. In such a case the roots must be compressed together. In one or two cases I missed a root, and so failed to get complete block.

Three methods of nerve block have been employed.

(a) *Pressure by rubber covered forceps* The forceps were provided with rubber covered jaws 5 mm wide, with parallel sides. The nerve, having been freed sufficiently from the surrounding tissues, was picked up in the forceps and pressure made until conduction ceased. Care was taken not to drag on the nerve. By this method very quick compression was possible, but no measurement of the pressure used could be obtained.

(b) *Pressure by band and weights* The nerve was freed sufficiently to allow a ribbon 4 or 5 mm wide to be slipped underneath. This ribbon or band was of the kind known as "double satin." It has a smooth surface on both sides and is strong enough to withstand stretching by small weights. This ribbon was soaked in warm saline before use. The length of the ribbon was about 10 cm and the two ends, after the ribbon was in position, were tied together by a strong thread, which then passed over a pulley fixed directly above the nerve. A hook was placed on the free end of the thread, so that weights could be applied quickly. Below the pulley, and fixed to the same stand, was a small hammer with a smooth rounded ivory head of the same cross section throughout ($r=7$ mm). This hammer head was slipped into position inside the ribbon loop and just touching the nerve. The weights were then hung on to the hook attached to the end of the thread passing over the pulley, and as the pulley and the hammer were both clamped firmly to the same stand, the nerve was compressed between the ribbon and the smooth surface of the hammer head without any dragging. This simple method of pressure proved very useful, as so little manipulation of the nerve was required, it was only necessary to free it from the surrounding tissues for about 10 mm. It did not give an absolute measurement of the pressure used, for necessarily a portion of the weight was taken by the rounded surface of the hammer over which the ribbon passed.

(c) *Air pressure method* In order to obtain a measure of pressure which would be absolute, a third method was devised. A pressure system was constructed by which the nerve could be compressed *in situ* by air pressure against a metal support. The pressure system included a mercury manometer, registering up to 1300 mm of Hg, a bicycle pump and a pressure box, the distal end of which was closed by a rubber membrane 4 mm in thickness.

The pressure box (B) was held in an outer metal case (A) into which it slipped easily,

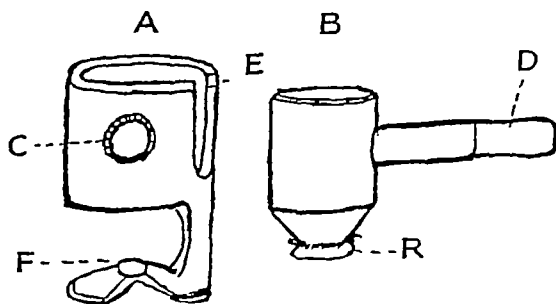


Fig. 1 Air pressure apparatus

and was fixed firmly in position by the screw (C) the tube (D), going to the manometer and bicycle pump, being engaged in the slot (E) The outer case (A) was furnished with a platform (F) on which the nerve rested. Two pressure boxes were used, one with a platform 6 mm. in diameter, the other 3 mm. in diameter The nerve was thus compressed over a definite length. Before use the whole pressure box was kept in warm saline, and a sheet of mesentery was spread over both the metal platform and the surface of the rubber membrane to prevent actual contact with the nerve When the nerve had been freed for a sufficient distance the platform (F) was placed underneath it care being taken not to stretch the nerve The outer case (A) was held in position by a clamp The pressure box (B) was slipped inside the outer case (A), the tube (D) engaging in slot (E) until the rubber membrane just touched the nerve lying on the platform (F) Then the screw at (C) was tightened, and the whole formed a closed system in which any increase of pressure was transmitted to the nerve through the rubber membrane Pressure was raised by using the bicycle pump, and the amount registered by the Hg manometer

It was found possible to keep up a pressure of 1000 mm. of Hg without appreciable leak, by using a rubber 4 mm. thick. Various other materials were tried, but none could withstand the pressure used, and even with this thickness of rubber there was an occasional burst if the pressure was raised much above 1000 mm. As the membrane was supported against the platform no force was wasted in distending the rubber The pressure could be raised to 1000 mm. of Hg in less than half a minute

In the frog and toad experiments, single break shocks from an induction coil were used, the kathode being placed toward the seat of compression and the muscle The shocks were sent in at regular intervals (5 or 6 seconds) and the contractions recorded on a slowly moving drum The threshold was first tested, and the stimuli used were either just maximal or just sub-maximal The electrodes (guarded wire 3 mm. apart) were placed as far as possible from the site of compression, so as to preclude escape of current around the area of block This is a very necessary precaution (Kato (7))

In the cat experiments artificial stimuli have not been used, but a conduction block has been applied to the natural respiratory impulses passing down the phrenic nerve The difficulties associated with the use of electrical stimuli have thus been avoided Moreover, the phrenic nerve has been subjected to a form of blocking to which it is liable in disease, *i e* compression by mechanical factors

Results

(a) *Measurement of pressure* The amount of pressure necessary to produce block varies considerably in different animals In large animals a greater compression is needed For example, in a medium sized cat a pressure of 1100 mm Hg applied to the phrenic nerve abolished conduction in 13 minutes, the excursion of the diaphragm being halve in $2\frac{1}{2}$ minutes But in a large old cat a similar pressure applied for

15 minutes produced a partial block only, the excursion being reduced to two-thirds. Since it was not possible to increase the air-pressure further a 5 mm band and weights were substituted, 1125 grm was then required to abolish conduction, a weight much in excess of the average requirement.

The criterion of complete block is either absence of movement on the affected side of the diaphragm, or a passive movement, transmitted from the chest and normal side. This passive movement is inverse to that of the control side (see Fig 2).

At the beginning of an experiment a tracing of the two sides of the diaphragm taken with a faster movement of the drum shows a completely synchronous movement of the two sides. As pressure is put on the nerve and conduction begins to fail, the excursion on the experimental side begins to grow less, and at the same time there is a progressive delay in the downstroke of the lever, indicating delay in the commencement of inspiratory contraction. As pressure is continued, delay becomes more and more marked until at length the two levers may move inversely. When this occurs paralysis of the experimental side is complete (Fig 2). The onset of delay varies in different animals.

As partial block was the condition aimed at in some experiments the pressure was not always maintained until paralysis ensued. Some figures will, therefore, be given showing the amount of pressure and time required to cut down the excursion of the one side to one-half.

These animals were medium sized cats (3-4 kilo), the large animal mentioned above being excluded.

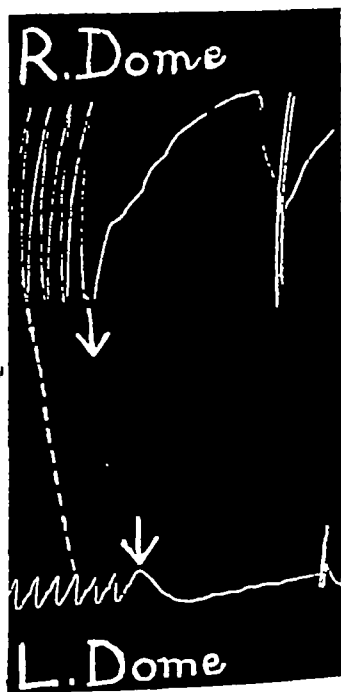


Fig 2 Right Dome Insp down Left Dome Conduction in Left Phrenic blocked by compression Left Dome is moved upwards passively by the chest during inspiration Arrows mark moment when the drum advanced by hand Shows inverse movement of the two domes White dotted line indicates simultaneous events

TABLE I. Pressure applied to phrenic by band and weights

Partial nerve block			Total nerve block		
Width of band (mm.)	Weights applied (gram.)	Time to cut down cont. to $\frac{1}{2}$ (minutes)	Width of band (mm.)	Weights applied (gram.)	Time to cause paralysis (minutes)
5	260	4	5	340	10
5	320	8	5	320	18
4	460	3	4	460	5
4	513	4.5	4	430	10
4	390	4			
4	280	6			
4	440	7			
4	360	3			
Average 380 gram. for 5 mins.			Average 357 gram. for 10½ mins.		

On an average a weight of just under 400 gram reduced the excursion to one-half in 5 minutes and abolished conduction in 10 minutes

These figures may be compared with the following obtained by the air-pressure method. In a medium sized cat a pressure of 1100 mm Hg applied to 6 mm. of nerve reduced the excursion to one-half in 2½ minutes, and the muscle was paralysed in 13 minutes. In another medium sized animal a pressure of 800 mm Hg applied to 3 mm. of nerve reduced the excursion to one-half in 7 minutes, and to one-third in 8 minutes.

The time factor is so important in these experiments that after these figures had been obtained, an attempt was made to produce the same effects by using a small pressure over a prolonged period. As an average weight of 400 gram. had abolished conduction in 10 minutes, a weight of 80 gram. was chosen and applied to the nerve in a medium sized young cat, by a 4 mm. band. Forty-seven minutes after the pressure had been applied the excursion was cut down to one-half and at the end of 1 hour and 20 minutes the excursion was very small, and the delay so great that the levers moved almost inversely. At the rate of progression conduction would probably have been abolished in one hour and a half.

This is the smallest pressure with which a complete experiment (*i.e.* conduction block, recovery, section of nerve to show complete paralysis) has been obtained. That smaller pressures would be effective is indicated by the following observations.

An air-pressure of 100 mm Hg applied for 50 minutes in a medium sized cat produced a diminution of excursion of 12 p.c. A pressure of 40 gram. applied by a 4 mm. band to the phrenic of a large cat (9½ lbs.) showed no change at the end of 1 hour, a slight diminution in 1 hour 20 minutes, and a diminution of 25 p.c. at the end of 2 hours, when the experiment was stopped. A pressure of 60 gram. applied for 2 hours

produced a block of 30 p c. The pressure being then doubled, a block of 70 p c was produced at the end of the third hour. A further 60 grm being added the block was complete at the end of another half-hour.

It was not always possible to use the pressure box on the phrenic, as the lower trunk given off from the brachial plexus is often extremely short, and it is difficult to place the box in position without stretching the nerve. The result of stretching the nerve is soon seen in the diminution of excursion of the lever before pressure is applied. When the air-pressure apparatus caused stretching the band and weights were substituted. This apparatus, however, is quite convenient when used on an isolated nerve muscle preparation. Experiments on gastrocnemius and tibialis anticus sciatic preparations taken from medium sized frogs gave the following results.

TABLE II. Frog

Pressure (mm Hg)	Length of nerve compressed (mm)	Time to abolish conductivity (minutes)
900-1000	3	4
1100	3	4½
1100	6	6
1100	3	8
930	3	8
1000	6	9
1000	3	9

Average 6.6 mins.

In three experiments where the pressure used was between 500 and 600 mm the average time to abolish conductivity was 7.8 minutes. In a group of nine experiments where pressures between 900 and 1100 mm were used, the average time taken to reduce the contraction to one half was 5 minutes.

There was considerable variation in the figures obtained from frog gastrocnemius-sciatic preparations, when the band and weights were used. Part of the variation was due to the fact that the weights were not applied at a uniform rate. In one group of experiments a weight of 453 grm was attached to a 5 mm band. The average time taken to abolish conduction was 1.9 minutes.

(b) *The loss of contraction and tone in the muscle.* When one phrenic is cut in the neck the lever attached to the ipsilateral dome at once ceases to fall and rise (although a small inverse movement may be apparent, as mentioned above). At the same time the level of the lever is raised above the former expiratory level. The muscle has lost its tone, and has risen higher in the chest. This can be confirmed by inspecting the

abdominal surfaces of the domes, and it will then be seen that the paralysed side is higher in the thorax than the healthy side

As conduction becomes blocked loss of contraction and loss of tone result progressively (Fig 3)



Fig 3 Cat Upper tracing, Right Dome Lower tracing, Left Dome Inspiration, down stroke Time in secs. Left Phrenic compressed by band (5 mm) and weights Total of 320 grams applied in 40 gram weights. Tracing is continuous Occasionally drum advanced by hand to show time relations

Contraction is cut down smoothly and gradually until there is a very small movement of the lever The more slowly the compression is carried out, the more smooth and gradual is the diminution in excursion There is no sign of a step-like diminution of contraction, or of sudden failure

Loss of tone is less apparent, because gradual, when the blocking is carried out slowly The most rapid and marked loss takes place when the nerve is compressed quickly by forceps Diminution of contraction can be demonstrated before loss of tone In the experiment in which a small pressure was applied for a long period (see Fig 4) it is quite clear that a diminution in excursion has taken place before any loss of tone After 16 minutes' pressure some loss of excursion is obvious, at the end of another 5 minutes the loss of tone is apparent

After this both contraction and tone diminish side by side If conduction has not been completely abolished, cutting the phrenic on the experimental side will produce a slight further loss of tone and movement, but if it has been abolished cutting the phrenic makes no difference to the tracing

Recovery takes place in the same smooth and gradual manner (see Fig 5) After a variable interval conduction is re-established and the muscle begins to contract again The fact that an improvement in contraction may be seen within a few seconds of releasing the pressure indicates that the mechanical constriction is the direct paralysing agent The first partial recovery of muscular contraction is always detected before any recovery in tone is apparent With an increased excursion

of the lever, the inverse movement gradually changes. There is a short period during which the levers move asynchronously, after which they rise and fall together.

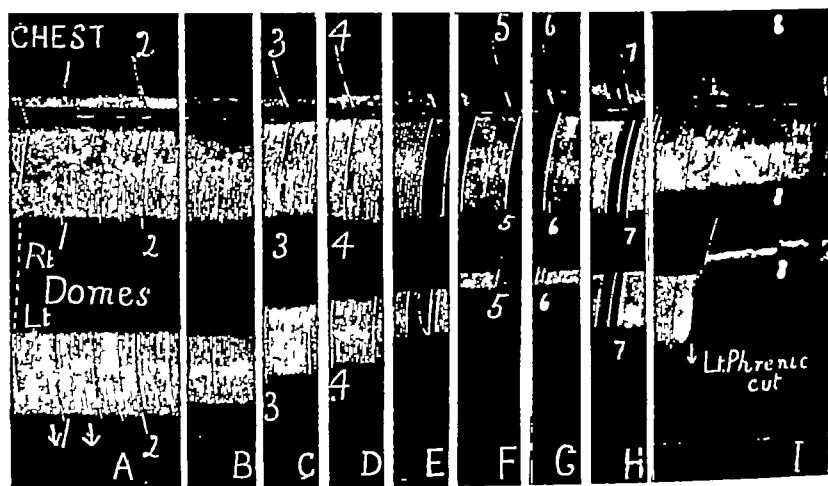


Fig 4 Cat. Chloralose. Upper tracing, Chest. Insp up. Lower tracings, Domes. Insp down. The single figures indicate simultaneous events and mark deep inspirations. Between the arrows on A a weight of 80 grams was applied to the Left Phrenic by a 4 mm band. Arrow on I marks cutting of Left Phrenic.

A, 11 53 a.m. Pressure applied to nerve. B, 12 8 p.m. Diminution of contraction. Slight rise of tone. C, 12 24 p.m. Distinct loss of tone. D, 12 30 p.m. Further loss of contraction and tone. E, 12 38 p.m. Contraction halved. Delay on left side. F, 1 5 p.m. Small movement. Further loss of tone and marked delay. G, 1 9 p.m. The nearly paralysed Left Dome is moved passively upwards by the chest, when big inspiration occurs (6), instead of completing its delayed inspiratory downward movement. 1 11 p.m. Pressure relieved. H, 1 25 p.m. Partial recovery of contraction. Slight delay. No recovery of tone. I, 2 21 p.m. Left Phrenic cut. Loss of contraction and tone. Inverse passive movement, accentuated when deep inspirations occur.

Pressure continued after the block has been established delays the rate of recovery. After recovery a block can be reproduced with a smaller pressure.

In the slow compression experiment quoted above the excursion of the experimental side began to improve very shortly after the pressure was released (see Fig 4). At the end of 4 minutes the excursion of the lever was doubled, and the two sides were moving nearly synchronously, with only a very slight delay on the affected side. No recovery of tone was observable at the end of 20 minutes. A rise was apparent after

three-quarters of an hour. At the end of an hour the experimental side was moving as well as before compression, relatively to the control side

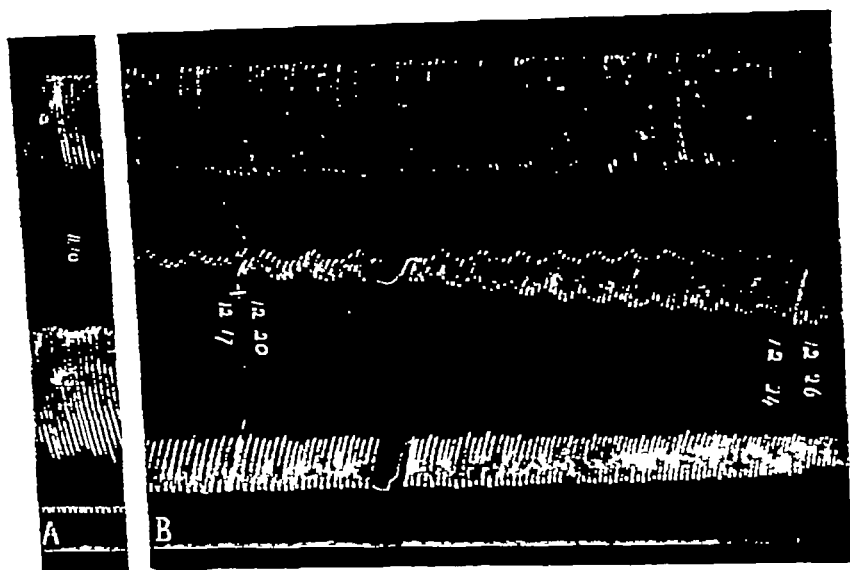


Fig. 5 Cat. Decerebrate. Curve showing recovery of conduction. Time in secs. Top tracing, Right Dome. Middle Left Dome. Lowest, Sternum. Inspiration downwards in each.

A, 11 10 a.m. Shows excursion of the three levers before dissection of Left Phrenic. Some alterations of levels between A and B. B At 12.7 p.m. conduction in Left Phrenic had been blocked by pressure for 40 seconds with rubber-covered forceps. Loss of contraction and tone followed. At 12.17 p.m. no improvement. Pause for three minutes at spot marked —. At 12.20 p.m. gradual progressive improvement of contraction seen, very slight recovery of tone. At 12.49 p.m. excursion of the two sides was nearly equal.

Tracing shows great increase of Chest movement when Left Dome is paralysed, and gradual diminution as conduction in Left Phrenic recovers. Oscillations in Diaphragm are seen fairly frequently and are not confined to the experimental side.

Respiration was slightly more shallow. Seventy minutes after the pressure was released, the exposed phrenic was cut. At this moment the experimental side was contracting as well as the control, but it was higher in the chest. Cutting the phrenic produced a further loss of tone, and a very small movement of the lever which was inverse to the sound side, i.e. passive movement. Conduction for motor impulses invariably recovers before those which control tone.

One point appeared unexpectedly. Before diminution of tone begins there is a slight preliminary rise of tone, which lasts for a variable

period dependent on the rate of compression. It is followed at once by a loss of tone. This rise of tone followed by a diminution can be seen in the tracings (Figs 3 and 4).

(c) *Conduction of stronger impulses through partial block.* The object of this part of the investigation was to see if a block, which hindered the normal respiratory impulses of an anæsthetised animal was impenetrable to the impulses sent down in hyperpnœa. Various methods of producing an increased depth of respiration were tried, CO_2 inhalation, afferent stimulation of sensory nerves, ammonia to the nostrils, freezing of vagi. These agents were tried during complete block, incomplete block and recovery from block. Partial block was attained by raising pressure until the excursion was cut down to one-half, and then releasing some of the pressure, so that the contraction remained fairly constant. Hyperpnœa was successfully produced by the inhalation of CO_2 or by Faradic stimulation to the femoral nerve.

Fig 6 shows the result of stimulating the afferent end of the cut femoral nerve for a few seconds, the left phrenic being in a state of partial conduction block.

The interpretation I would put forward of this result repeatedly obtained is as follows. When there is an increased discharge from the respiratory centre the chief adjustment occurs in the chest. The sound

side of the diaphragm responds by descending further with each inspiration, and increasing its contraction. What happens to the experimental side? If the nervous impulses of hyperpnœa can get through a

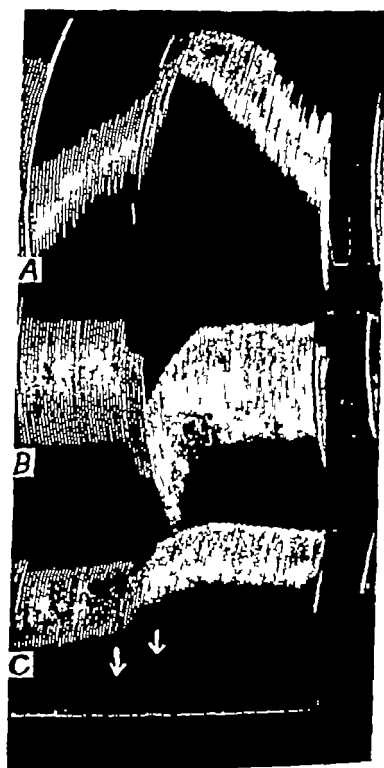


Fig 6 A, Chest Insp up B Right Dome. Insp down. C, Left Dome Insp down Faradic Stim to Aff Nerve between arrows Time in secs Partial conduction block in Left Phrenic

Faradic stimulation produced an increased rate of respiration, large inspiratory increase in chest (not fully seen) increased inspiratory tone followed by increased excursion in Right Dome. The Left Dome is pulled upwards by the increased chest action.

partial block, then the affected side should behave like the control side and descend. If the impulses already partially ineffective at the "block" remain so still, the enfeebled muscle, unable to withstand the increased upward aspiration from the chest, takes up a more expiratory position and shows smaller excursions, in short, it behaves more like a paralysed muscle.

This explanation is supported by an experiment in which the animal took occasional deep inspirations of its own accord (see Fig 4). In the first part of the tracing these deep inspirations appear in the diaphragm records as downward movements, in the chest as an upward movement. Later on these deep inspirations appear to affect the chest only, the domes being unaffected. When a partial degree of block has been established on the experimental side (as shown by the diminution of movement and tone, and delay in action) a deep inspiration shows a big movement of the chest, no change in the healthy dome, but an upward movement on the affected side (section G, Fig 4). The moment has arrived when the increased action of the chest is able to mask the feebly acting muscle, and pull it upwards with inspiration. The reverse is seen during recovery. Ten minutes after the pressure was released the excursion had attained half its original amount, and deep inspirations no longer appeared as upward movements on the blocked side. The contraction was sufficiently strong to overcome the upward pull of the chest. With further recovery deep inspirations appeared as downward movements on the experimental side, the excursion and behaviour of the two domes being identical. Conduction for motor impulses having been fully restored, the phrenic was cut. The signs of a paralysed muscle are seen immediately, loss of tone, almost complete loss of movement, the small passive movement being inverse to that of the sound side. A deep inspiration now showed as a downward movement on the sound side, but as a large upward passive movement on the paralysed. All the stages from partial block to complete paralysis are illustrated in this figure by the behaviour of the muscle during deep inspirations.

The conclusion is drawn that the impulses of hyperpnoea are no more successful in penetrating a region of partial or complete conduction block than are the impulses of ordinary respiration.

(d) *Histology* Many of the nerves compressed were examined histologically. The nerve, still subjected to pressure, was cut out of the body, and the pressure was not released until the nerve was fixed. Staining by Marchi and Weigert-Pal showed very little change. There was a general diffuseness of staining in the compressed area, but nothing conclusive.

Discussion The amount of pressure which can be exerted on a nerve by glands or new growth or fibrous tissue is obviously unknown. Air pressure of 1000 mm Hg will abolish conductivity in a phrenic or frog's sciatic in a few minutes (4/13). So, similarly, does a weight of 400 gm applied to the nerve by a 4 mm band. A weight one-fifth of this requires about one hour and a half. Pressure of 100 mm of Hg applied for 50 minutes reduced excursion by 12 p.c. Since fibrous tissue or new growth may exert pressure over periods of weeks or months, it may well be that such pathological conditions may block conduction with pressures considerably smaller than those above quoted.

The figures obtained by former workers on sciatic preparations have varied considerably. Ducceschi⁽⁸⁾ obtained loss of conduction in 1 minute by using a thread 3 mm wide and attaching 20 gm. This would be equivalent to a weight of 340 gm attached to a 5 mm. band. This figure is very close to the weights used in my frog experiments.

Meak and Leaper, using pressure transmitted by oil, give high figures, 2000 mm of Hg for 7.5 minutes, if the assumption that their pressure of 40 lbs is referable to the square inch be correct. The discrepancy is probably explained by the fact that I used either just maximal, or just sub-maximal stimuli, and also that I endeavoured to avoid the possibility of spread of current around the area of block. Meak and Leaper make no mention of the position of their electrodes and they used fully maximal currents. One of Ducceschi's results is probably explained by this question of spread of current. He noted that when he compressed the nerve lightly, and stimulated (a) near the block, (b) near the central end, then conduction ceased earlier from the more distant point of excitation.

The argument for the all-or-none theory of nervous impulses is largely based on the failure of impulses set up by stronger stimuli to pass through a region of block which is impenetrable to impulses set up by a just maximal stimulus, i.e. a stimulus which just excites all the nerve fibres. These experiments on the diaphragm are compatible with this theory as the impulses of normal breathing and of hyperpnœa are extinguished at approximately the same time. There are, however, certain difficulties in the application of the all-or-none theory to these experiments, which will be dealt with in a later communication.

SUMMARY

1 The amount of pressure required to block conduction of respiratory impulses in the phrenic nerve varies inversely with the duration of application. A weight of 400 grm applied to 4 mm of nerve will block in 10 minutes, a weight of 80 grm applied to the same length, in one and a half hours

2 Conduction of motor impulses is impaired before conduction of the impulses which control tone

3 The muscle is paralysed from a functional point of view before complete loss of tone occurs

4 Recovery of conduction for motor impulses occurs first, and is much more complete (under the time limits of experiment) than recovery of tone impulses

5 The impulses conducted by the phrenic in hyperpnœa are no more successful in passing a region of pressure block in that nerve than are the impulses sent out in quiet respiration. This failure is compatible with the theory of all-or-none conduction in nerve fibre

The experimental work has been carried out in the Physiological Laboratory of the London School of Medicine for Women, by permission of Prof Winifred Cullis, to whom I am greatly indebted for her unfailing kindness and hospitality

My thanks are also due to Mr William Lofts, who has given me much assistance in the experiments

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THE INFLUENCE OF LENGTH ON THE RESPONSES OF UNSTRIATED MUSCLE TO ELECTRICAL AND CHEMICAL STIMULATION, AND STRETCHING

By F R WINTON

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Introduction The tonic and rhythmic activities of smooth muscle have distracted attention from the simple responses to stimulation approximating more closely to those of striated muscle. In the present enquiry some of the factors influencing the contraction of unstriated muscle under the simplest obtainable conditions are considered, with a view (a) to bringing under control the conditions which make for more accurate quantitative data, and (b) to defining the limits of a possible analogy between the contractile mechanisms of striated and unstriated muscles.

Two difficulties have to be overcome first, the anatomical fact that most smooth muscles are formed of a large population of small cells arranged in an irregular network, and that contraction may be associated with a sliding movement between the cells (Grützner(1)), second, physiological variations of tonus and liability to spontaneous contraction. The correlation between the properties of a muscle and those of its constituent fibres is indeterminate unless the fibres are parallel and longitudinally arranged. This anatomical arrangement obtains in the dog's retractor penis, which formed the material of most of the experiments described below. If such a muscle be stimulated under isometric conditions, sliding movements between the cells are extremely unlikely. Fortunately for the present purposes, this muscle in isolation is relatively free of tonus(2), and in a considerable proportion of preparations shows little or no tendency to perform spontaneous movements. It is likely, therefore, that the mechanical properties of such a muscle will be relatively simple functions of the chemical and energetic changes associated with its activity.

Method Adequate electrical stimulation of smooth muscle presents certain difficulties, chief among which are, first, the relatively strong currents required together with the injury which follows unduly intense stimulation, and second, the factors preventing uniform excitation and

simultaneous response of a large population of cells. The second circumstance is of greater importance in isometric than in isotonic contraction for, if a contraction begins at one part of a muscle before another part the former will shorten and the latter lengthen, while the lever gives little indication of the tension a fibre might develop at approximately constant length. This effect is greater if, as Brücke⁽³⁾ and Botazzi⁽⁴⁾ claim, the conduction of the excitation wave in the retractor is relatively slow (1-7 mm per sec) than it is if de Zilwa's record⁽⁵⁾ of simultaneous response of two halves of a muscle to stimulation of one-half be accepted. This consideration is reinforced by analogy with other smooth muscle, and by Brücke's⁽³⁾ observation showing the difficulty of obtaining monophasic action currents in this muscle (cf Adrian⁽⁶⁾). Such imperfect continuity of a muscle with respect to an excitation wave would again emphasise the importance of stimulating all the cells at the same moment.

Electrical stimulation in a moist chamber yields excellent responses, but tends to induce irreversible change in a retractor, which disturbs the long succession of constant responses to similar stimuli necessary for quantitative experiments. Consequently a stimulation chamber was employed, which consisted of a tube, about three times the cross-section of, and about the same length as a relaxed muscle, opening at each end into a larger tube. The lower end was sealed save for openings allowing ingress of fresh and egress of used solution, a hooked capillary tube for attachment of one end of the muscle and flow of oxygen through its fine orifice, and silver ribbon formed inside into a spiral. The open upper end

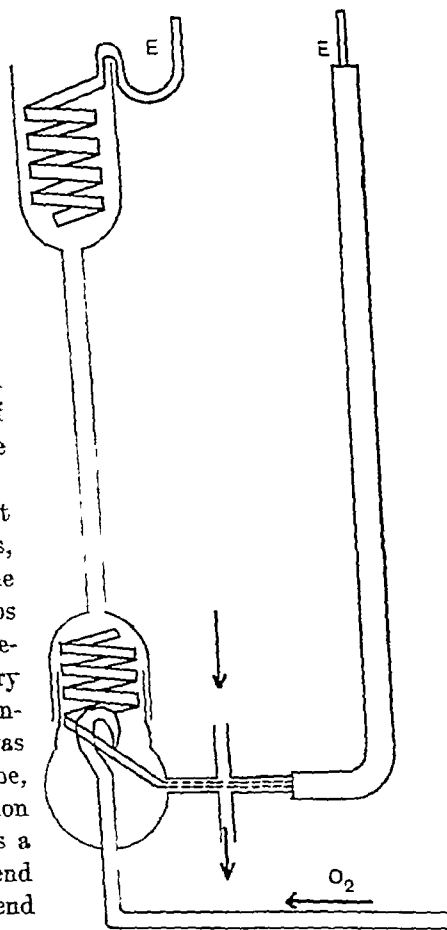


Fig 1 Stimulation chamber
E, E, wires to electrodes

contained a similar silver spiral. The thread from the upper end of the muscle passed along the axis of the upper electrode to the lever. Thus the current density was increased along the constricted region where the muscle was situated, and as both tube and muscle were of uniform cross-section, the potential gradient along the muscle was also fairly uniform. Moreover, the muscle never came into contact with the electrodes and was not therefore exposed to the electrolytic disturbances apt to occur in their vicinity. The electrodes were heavily coated with silver chloride when direct currents were used, and sometimes also when faradic stimulation was employed. In the latter case some rectification of current occurred when the electrodes were bright, owing, as A V Hill(7) pointed out, to their unequal size and dirtiness, the direct current produced appeared to be too small to affect the result. The apparatus was immersed in a large water-bath maintained at a constant temperature.

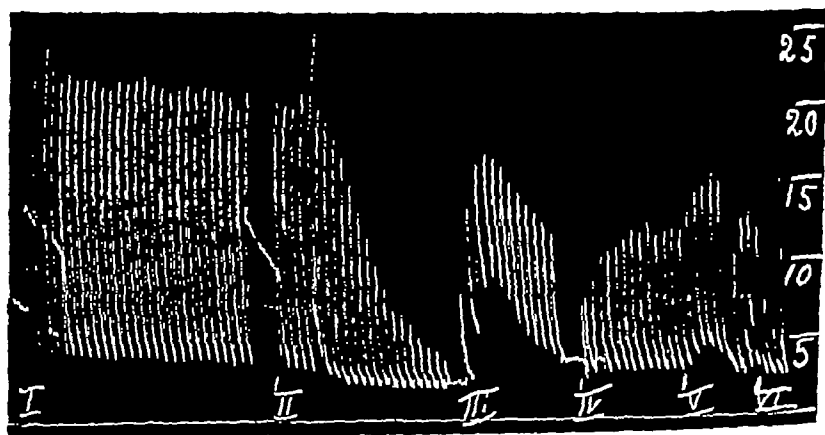


Fig 2 Isometric responses of a retractor penis (dog) in Burn and Dale's solution at pH 7.6, 35° C. Faradic stimulus, 5 sec duration, applied once a minute. Scale of tension in gm wt. on right. I-II, about 30 sec oxygenation between stimuli, II-III, no oxygenation, III-IV, oxygenation, as in I-II, IV, 1 hour's rest, with continuous oxygenation, IV-V, same as I-II, V-VI, sodium bicarbonate solution added, oxygenation continued, VI, return to normal solution, as in I-II.

A continuous stream of fine oxygen bubbles passed up the tube. It was interrupted just before stimulation until about 75 per cent relaxation. The period of such interruption was not a critical factor influencing responses to stimuli applied every 5 minutes, as was the custom in these experiments. If, however, stimuli were repeated frequently it became

an important factor Fig 2 shows isometric responses to faradic stimuli applied every minute They are somewhat irregular owing to slight variation of duration of oxygenation The effect of complete absence of oxygenation with its concomitant massage of the muscle is shown at *II*, which is soon followed by a steep fatigue curve The strikingly incomplete recovery when oxygenation is re-started is also shown

Phosphate-buffered solutions were not found to allow undiminished responses over long periods A carbonate buffered solution, of the composition described by Burn and Dale(s), was therefore used, and an attempt made to reduce the effective changes of hydrogen-ion concentration by replacing the solution immediately before each stimulation

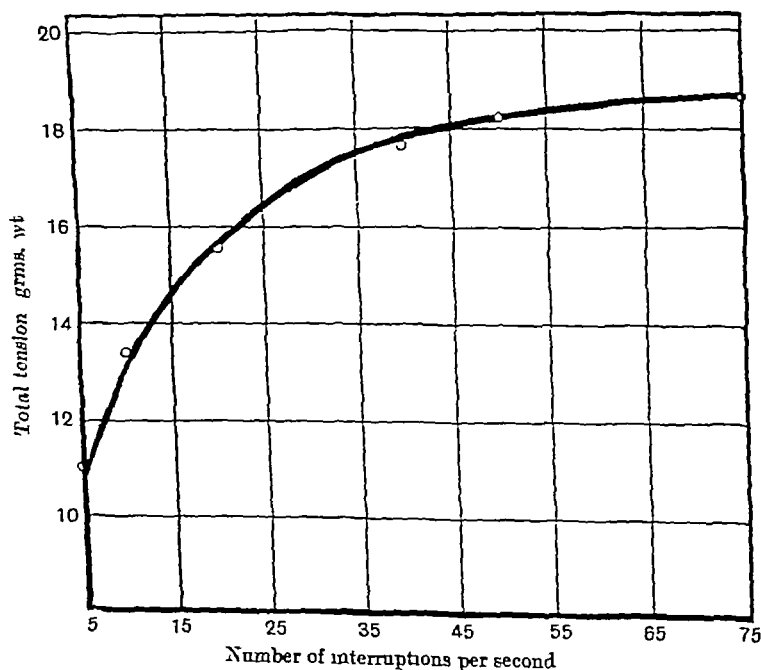


Fig 3 Tension developed by retractor penis as a function of frequency of interruption of a faradic stimulus of 5 sec duration. Points on the curve are the means of values obtained at increasing and decreasing frequencies respectively

The reputation of smooth muscle for capricious response suggested an examination of the relation between tension developed and the strength, frequency, or duration of the stimulus, whether galvanic or faradic no discontinuity was found The influence of frequency of faradic stimulation is shown in Fig 3 the curve allays the suspicion that certain

frequencies might produce inhibition, and records a response increasing with frequency of interruption up to 75 per sec in a tissue with slow general time relations Fig 4, showing the influence of temperature, indicates continuity and the region of optimum response Increasing temperature induces, within limits, increased responses, as is the case with tetanic contraction of striated muscle Associated variation of initial tension, however, may here contribute to modification of the responses

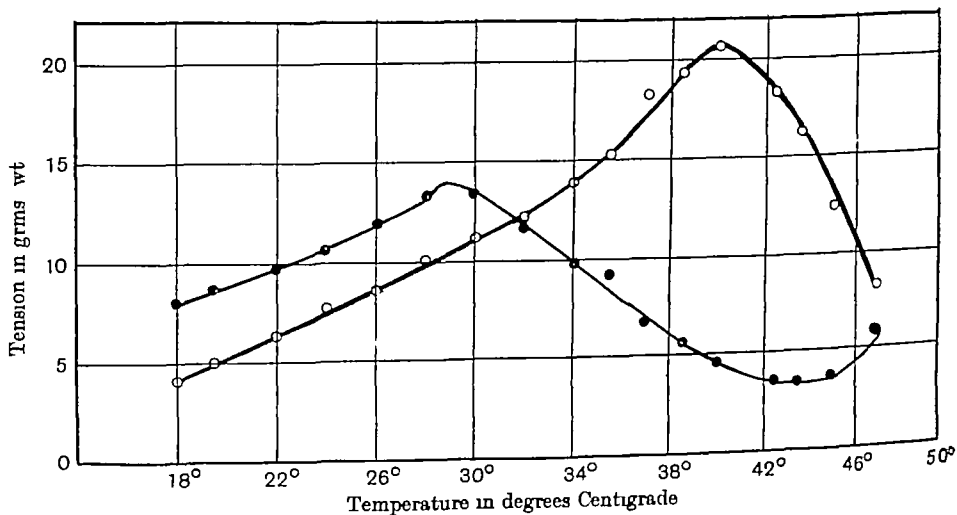


Fig 4. The influence of warming (i) on the initial tension (dark points), and (ii) on the tension developed on faradic stimulation (circles) (Retractor penis, stimulated for 3 sec every 5 minutes.)

Tension levers of various strengths were used One with a long light bamboo pointer, giving about 1 cm excursion for 10 grm wt was found convenient It allowed shortening of the order of 1 mm in a full contraction Observations were made on the reduction of response, when a muscle was stimulated at a particular length, caused by shortening at various uniform velocities They indicated that truly isometric tensions would exceed those recorded by such a lever by 5-10 p c

The influence of initial *length* of muscle on its response is discussed later The independent influence, however, of initial *tension* also is of importance in smooth muscle It is difficult to isolate changes of initial tension from concomitant changes of environment, but at a given length, increase of initial tension appears to reduce the response to stimulation in experiments involving the slow fall of tension after a

sudden stretch, or the rise of tension due to chemical or electrical stimulation

Spontaneous contractions, slow variations in tone, and simple responses to stimulation, retain about the same quantitative relations, whether manifested as changes of isometric tension or of isotonic length

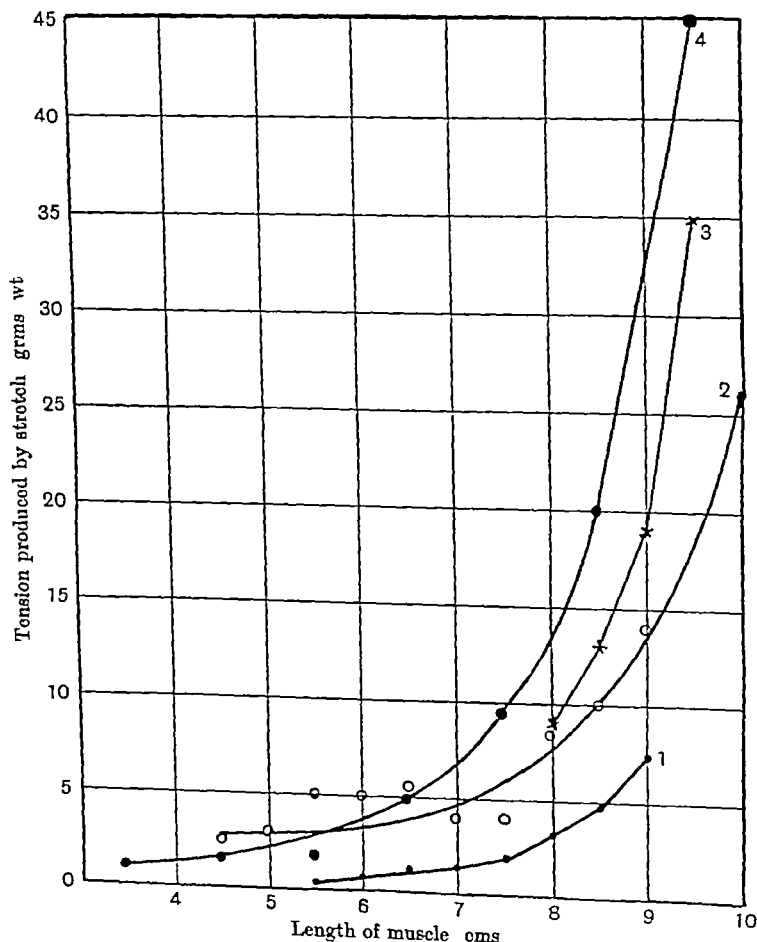


Fig 5 The tension produced by sudden stretching at varying lengths of four different retractors. Curves 1-4 represent stretches of 1, 2, 5 and 10 mm. respectively (Muscle 4 was longer than the others and 3 cm has been subtracted from each value of its length for inclusion in the figure)

The influence of length on the reaction to stretching The curves shown in Fig 5 give a rough indication of the relation of longitudinal stress

to strain at varying lengths of the unstimulated muscle. A crude technique only was employed, which involved rapid lengthening of the muscle by turning a handle manually. The tension produced varied with the speed of stretching, which was kept fairly constant, by adopting a rhythmical procedure regulated by metronome.

The curves derive from experiments on different muscles, and are not reduced to a standard length. They show a general resemblance, within the region shorter than the length of maximal response to stimulation, between the effects on the tension (i) of stretching, and (ii) of stimulation. They demonstrate, further, the increase of longitudinal elasticity with length. This is again shown, on a slower time scale, in the lower tension-length curve shown in Fig 8, where the slope increases with length. The muscles were in each case stretched during a period of $\frac{1}{5}$ – $\frac{1}{2}$ second, maintained at the greater length for 10 secs, and returned to the original length. Successive reactions to the same stretch showed no significant variation, if the initial length, and the rate of stretching were kept constant.

The retractor penis is less readily excited by mechanical disturbance than most plain muscles. It will, for example, in the circumstances obtaining in these experiments, show no contraction superimposed on a sudden stretch or after pinching with forceps.

The influence of length on the response to stimulation. A defect of the apparatus described above is that an electrical stimulus necessarily varies with the length to which the muscle is stretched, owing (1) to a reduction of the proportion occupied by the muscle of the cross section of the tube, (2) to increase of the length of the potential fall, (3) to reduction of muscle substance directly stimulated, and introduction of phenomena due to slow conduction of excitation, if some of the muscle inadvertently emerges from the narrow to the wider portion. Analogous errors affect stimulation in a moist chamber, or by the application of drugs. It was not found possible to circumvent all these defects. An attempt was made to prevent them from vitiating the results by using a variety of tubes, and by comparing responses to electrical and chemical stimulation. The results as a whole suggest that the inevitable errors are not large enough to mask the essential correlations.

The muscle was attached at its lower end to the stimulation chamber as described above, and the whole of this chamber was fixed by clamps to a stage, the vertical movement of which was controlled by a pulley connected through gearing to an electric motor. The velocity of the vertical movement could be adjusted and measured. The upper end of

the muscle was attached to a stationary tension lever recording on smoked paper. In earlier experiments, variation of length of the muscle was achieved by moving the lever 0.5 cm. at a time, as in the work on striated muscle, but such sudden stretching or shortening produced large changes in tension. These were transient, but disturbed the state of equilibrium, interrupted only by stimulation, in which consistent experimental results were best obtained. The rate of change of length

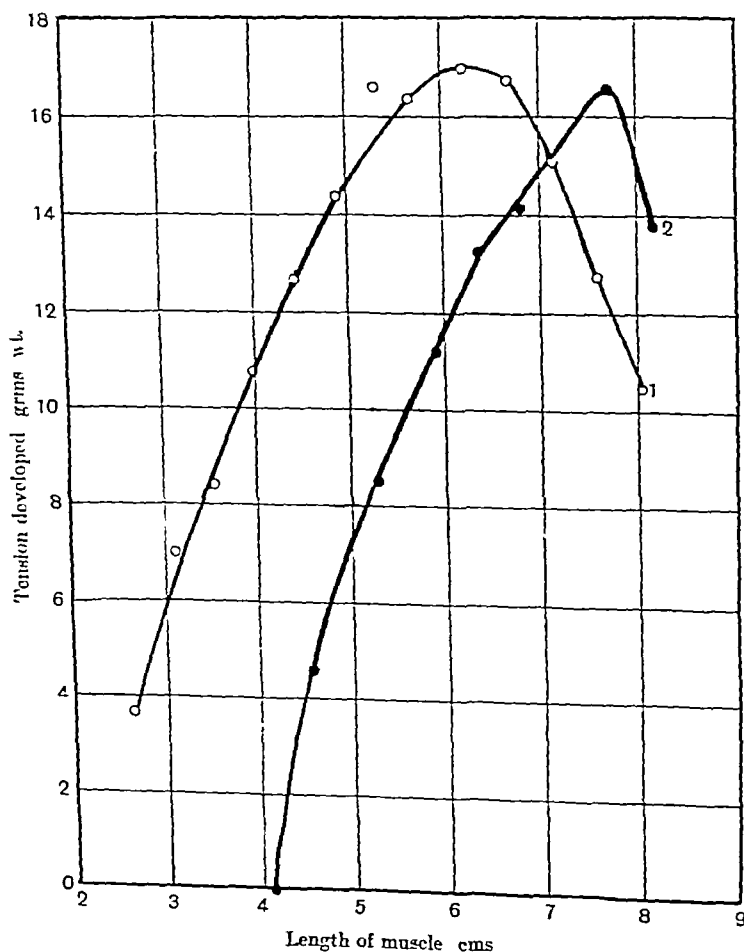


Fig 6 Tension developed by retractor penis when stimulated at different lengths. Curve 1, muscle stretched at 5.4 cm. per hour (circles) curve 2 muscle allowed to shorten at 5.4 cm. per hour (dark points) Faradic stimuli, 5 sec. Dale's solution, 36° C 40 hours since death of dog

varied from about 5 to 8 cm per hour in different experiments, and the reduction or increase of tension developed on stimulation, due to the continuous change of length, was a measurable experimental error, usually of the order of 1 p c

Eight retractors were examined, each at a series of different lengths. Galvanic and faradic stimuli of all durations gave similar results. Fig 6 shows a typical example of responses to a submaximal faradic stimulus, applied at intervals, while first stretching a muscle to beyond its optimum length, and then releasing. The irreversible increase of functional length of the muscle due to over-stretching is evident. Fig 7 shows responses both to electrical and to chemical stimulation, during (1) the release following an initial stretch, and (2) a second stretch. The maximum tension for stimulation with adrenalin is at a greater length than that for electrical stimulation and the curves for the former are steeper than those for the latter, differences to which little significance should be attached in view of the errors involved in the method. It will be noticed that the tension developed is not a linear function of the length, though the total tension exerted by the stimulated muscle approaches more closely to this simple relation, as shown in Fig 8.

The property of unstriated muscle described in this section has an interesting application to the behaviour of the parturient uterus, for it has seemed a curious fact that the muscle of the fundus and cervical canal should be in mechanical equilibrium both before and after expulsion of the foetus, and that in certain mammals such as rats, the thin distended portions of the uterus should be capable of expelling their contents against the resistance of adjacent stouter portions of the muscle. The profound influence of extension upon the tension which a muscle exerts in response to a sudden stimulus may again be seen in the responses of the guinea-pig's uterus to pituitary extracts, shown in Fig 9. In order more vividly to illustrate the sufficiency of this mechanism, a horn of a guinea-pig's uterus was attached by its apex to a lower rigid support, while threads were connected to its centre and to its vaginal end, and attached each to a light isotonic lever. The load on the central lever was now increased, so that the ovarian half only of the uterus was stretched for about an hour. The load was then again reduced to its original value, and about half an hour allowed for equilibrium to be established. The lever connected to the vaginal end was then fixed rigidly, so that the length of the entire uterus was kept constant, but any movement of the central segment could be observed. Pituitary extract now induced considerable movement of the central segment, in the sense of shortening

of the previously stretched half This effect was reversible, and elicited by successive doses of the extract

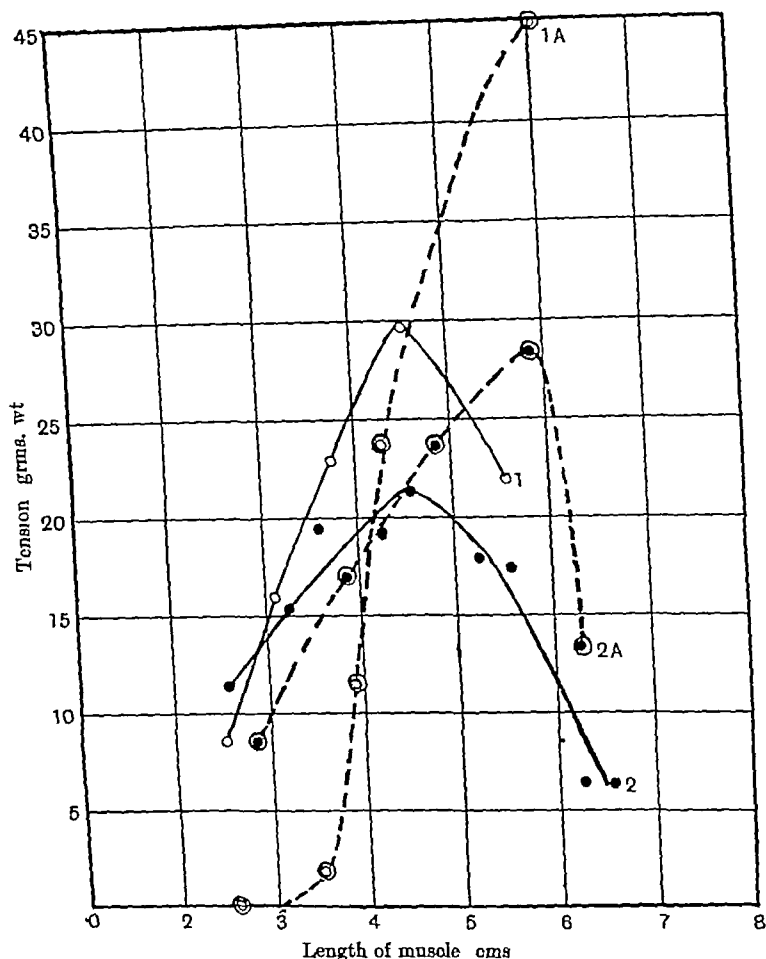


Fig 7 Tension developed by retractor penis when stimulated at different lengths. Curves 1 and 1A shortening at 4 cm. per hour, after a previous stretch (circles) curves 2 and 2A subsequent stretching at 4 cm. per hour (black points) Continuous lines 1 and 2 show responses to faradic stimulation, broken lines 1A and 2A those to 1 in 10^6 adrenalin

It may be recalled that increasing extension itself tends to produce more frequent auto-excitation (Brucke(3)), and increased response to stimulation with stretching might therefore be due to some form of summation of stimuli Chemical or other stimulation preceding a

superimposed electrical stimulus at a given length tends, however, to reduce the response to the latter, and the influence of excitation due to stretching should thus be in the sense of reduction of responses to stimuli applied at increasing lengths

The range of length within which muscle responds Smooth muscle develops effective tension on stimulation throughout a great range of length. Indeed, while skeletal muscles are often described as functionally almost isometric, unstriated organs approach more closely to isotonic

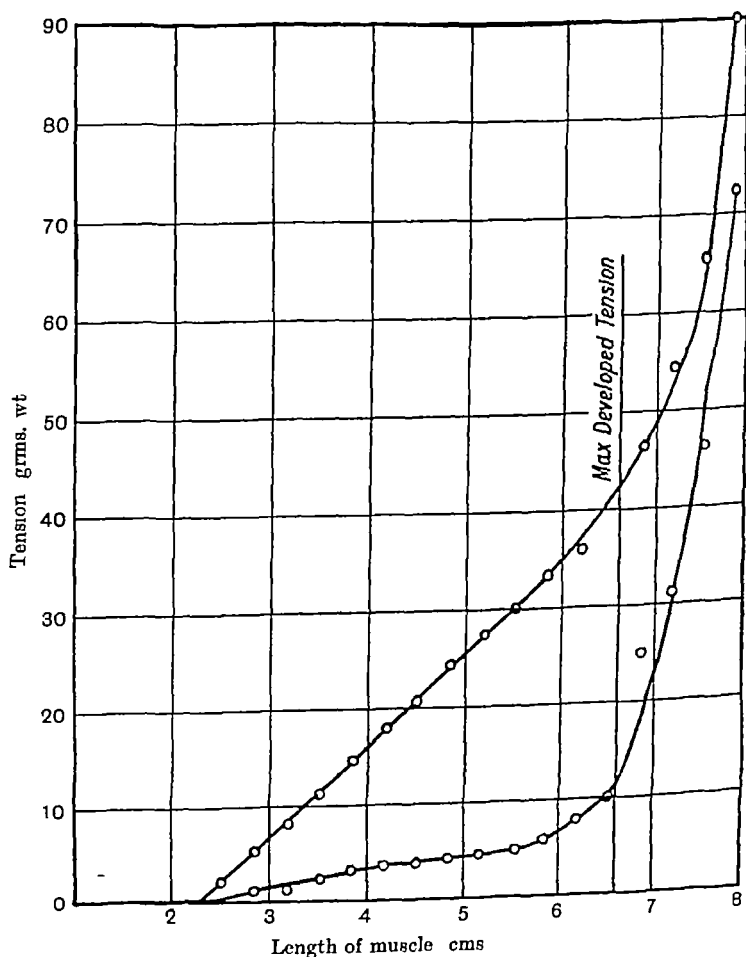


Fig 8 Tensions at various lengths of a stimulated (upper curve) and unstimulated (lower curve) retractor penis, stretched at 4 cm per hour. Faradic stimuli, 5 sec duration applied every 5 minutes at 36° C. This is the first muscle of Table I

conditions. It seemed interesting, therefore, to define this characteristic quantitatively.

A muscle stretched beyond its length of maximal response undergoes irreversible changes, as shown in Fig 6. It is therefore more expedient experimentally, and probably of greater physiological significance, to confine measurements to lengths shorter than this value, and to adopt this length as the standard natural length. If a muscle be stimulated at different lengths, and the tension of stimulated and unstimulated muscle respectively be plotted against the length, as in Figs 8 and 9, the ratio $W_m/T_m l_m$ (where W_m is the area between the curves up to the length of maximal response, T_m is the maximal tension developed at the optimal length l_m) is dimensionally a number, and may conveniently be taken to represent the relative range of effective response. Fig 8 shows the variation of tension with length of a retractor penis (i) unstimulated, and (ii) electrically stimulated. Fig 9 gives similar particulars for a guinea-pig's uterus stimulated with pituitary extract.

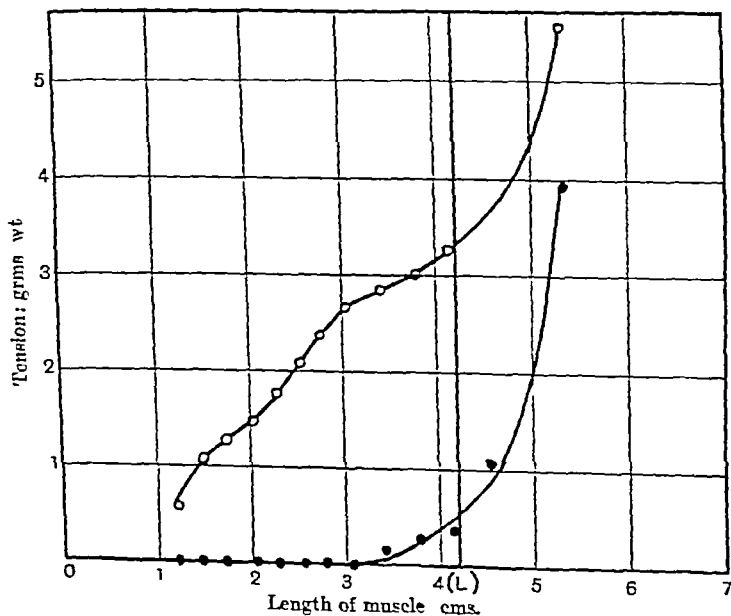


Fig 9 Tensions at various lengths of a stimulated (upper curve), and unstimulated (lower curve) uterus of guinea pig, stretched at 2.2 cm. per hour. Stimulus = maximal dose of post pituitary extract. Burn and Dale's solution, 37°C.

The table gives values for a number of muscles treated in much the same way—all but the uterus consisting of parallel fibres and being

electrically stimulated. The rectus abdominis of the frog was chosen as representative of striated muscle because it resembles smooth muscle in some of its properties, and notably in its considerable capacity of changing its length without great change of tension in an intact animal. This muscle, therefore, seemed likely to reveal continuity between the two classes of tissues if such existed. Values for its range of effective response are given in Table I, which, though somewhat lower, do indeed approach those given for smooth muscle. The classes of tissue defined by cross-striation and by short range of functional length, respectively, are therefore probably not co-terminous. Considerable reduction of this range of activity takes place in the course of a long experiment, it exceeds the associated diminution of tension development.

TABLE I.

Muscle	Area W_m gm. cm.	Tension T_m gm. wt.	Length l_m cm.	$W_m/T_m l_m$
Retractor penis (dog)				
(1) Stretched 4 cm./hr	68.3	31.0	6.6	0.33
Retractor penis (dog)				
(1) Stretched 5.4 cm./hr	46.3	17.0	6.2	0.44
(2) Released 5.4 cm./hr	36.9	16.6	7.6	0.29
Retractor penis (dog)				
(1) Released 8.4 cm./hr	65.2	15.8	9.7	0.42
(2) Stretched 8.4 cm./hr	40.2	13.0	8.3	0.37
Rectus abdominis (frog)				
(1) Stretched 10.0 cm./hr	10.0	9.15	3.7	0.30
(2) Released 10.0 cm./hr	11.5	14.3	3.9	0.21
Uterus (guinea pig)				
(1) Stretched	5.5	2.9	4.2	0.45

l_m is that length at which maximal development of tension occurs. T_m is the maximal developed tension, W_m is the area of the tension length curve up to length l_m .

The relation between the area (W_m) mentioned above, and the theoretical maximal work deserves brief attention. Experiments on the theoretical maximal work in striated muscles differ from those here described, in that stimulation in the former is usually carried out at the same initial length. In the tetanic contractions employed by Hill⁽⁹⁾, the muscle shortens and develops its maximal tension at the new length. The relation between length and response may be affected, as in the above experiments, either by the contractile or excitatory mechanisms, or by the actual change of effective stimulus associated with change of electrical resistance of the shortened muscle. The use of supra-maximal stimuli in striated muscle obviates errors due to the latter consideration, whereas the inevitable use of submaximal stimuli with easily damaged smooth muscle emphasises its importance. The instantaneous stimuli employed

by Meyerhof(10), on the other hand, ensure constancy of excitation, but tensions developed at different lengths then represent different, and not necessarily maximal, points along the isometric tension-time curve. Losses of energy due to internal friction thus reduce the tension measured. This conception of the theoretical maximal work which a muscle can perform in response to a particular stimulus does not, however, imply supra-maximal stimulation, and experiments are in progress intended to determine the quantity for smooth muscle in this way. In the meantime it may be mentioned that the areas measured above for the retractor penis, if taken as of the same order as the heat production of the muscles, would correspond to a rise of temperature of the order of 0.003°C . Such an area, however, represented about six times the actual mechanical external work done in shortening at a favourable uniform velocity in a particular muscle which developed the same isometric tension.

SUMMARY

1 A long succession of constant responses under approximately isometric conditions can be obtained with the retractor penis in the stimulation chamber described.

2 The influence of a number of factors on the tension developed was examined, and the best conditions for uniform responses determined.

3 The longitudinal extensibility of unstimulated smooth muscle decreases with length.

4 The isometric tension developed in response to electrical or chemical stimulation increases with length up to a maximal value, and then decreases. The total tension of a stimulated retractor approaches a linear function of the length more closely than does the tension developed.

5 The mechanism of expulsion of a foetus by the uterus is discussed.

6 The range of length within which muscles respond effectively to stimulation is considered, and found to be of the same order in certain striated and unstriated muscles.

7 The theoretical maximal work of smooth muscle is considered in relation to measurements of tension development at different lengths.

I am indebted to Professors A. J. Clark and A. V. Hill for advice, and to Prof. E. H. Starling, Dr. Anrep, and others, for their courtesy in allowing me to excise the muscle from animals used by them.

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THE ACTION OF PITUITARY EXTRACT UPON THE PREGNANT UTERUS OF THE RABBIT

By H H KNAUS

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DIXON and MARSHALL⁽¹⁾ have recently shown the existence of a relation between the cyclic activity of the ovary and the pituitary gland. They state that the pituitary gland secretes into the cerebro-spinal fluid, and that the amount of secretion depends largely upon whether corpora lutea are absent or present, that is to say, the secretion of the ovary in the absence of fully formed corpora lutea has a specific stimulating effect in promoting pituitary secretion. Finally, they came to the conclusion that, as the corpus luteum is supposed to undergo retrogression shortly before the close of pregnancy, the revival of the ovarian secretion causes a sudden increase of pituitary secretion, which, by its action on the more irritable uterine muscle, brings about labour. Thus, it is the increase of the pituitary secretion that is looked upon as an important factor in starting parturition.

Approximately at the same time Clark and Knaus⁽²⁾ studied the conduction of contractions in the uterine muscle, and found that distinct changes in the mechanism of this muscle take place in the different stages of the œstrous cycle. The experiments were carried out on the isolated rat's uterus, and the tracings were obtained by recording simultaneously the contraction of three parts of the organ, namely, the vaginal, the middle and the ovarian parts.

It was found that in the diœstrous period there is a slow conduction between the ovarian and the middle part of the uterus, and that the vaginal part contracts independently of the other two.

A remarkable change takes place under the action of pituitary. All three parts of the muscle now contract practically simultaneously, just as they do in the normal uterus at œstrus or in early pregnancy.

It seems that pituitary has induced the same effect in the uterus at the diœstrous period as that which occurs naturally at œstrus and during early pregnancy.

These two independent observations suggested that the change of

the mechanism in the uterine muscle that occurred from the diœstrous period to œstrus and pregnancy might be due to an increase of pituitary secretion during the latter periods. That is to say, the changes here described in the behaviour of the uterine muscle from period to period might be due only to a varying increase and decrease of pituitary secretion, and in pregnancy they would be due to a steady increase, or even as Dixon and Marshall suggest, to a sudden increase of pituitary secretion at the end of pregnancy, finally bringing about labour. If this should be so then it seemed reasonable to assume that it might be possible, by creating these conditions artificially, that is, by injecting an appropriate quantity of pituitary into the body of the animal, to induce parturition at any time of pregnancy.

For this purpose it was necessary to keep the animal under an even influence of pituitary throughout a spell of 10 hours or so, in order thereby to create conditions similar to those which occur naturally at the end of pregnancy. It has been shown by the author elsewhere⁽⁷⁾ that a small quantity of pituitary extract (equal to 1 mgrm of moist posterior lobe), when injected intravenously into a cat, produces a marked effect on the uterus lasting for at least an hour. This indicated a method for keeping the animal under the prolonged action of a known quantity of pituitary by injecting the drug intravenously at hourly intervals.

Because of two obvious advantages the rabbit was chosen for this investigation. First, the rabbit's ear-veins offer the greatest possible convenience for repeated injections without injury to the animal. Secondly, if the doe is in good condition, she never ceases to breed, which consequently allows the investigation to be continued without interruption. Besides, the rabbit may be mated at any time during a prolonged heat, and, as the act of successful copulation is quite definite, the precise time at which pregnancy starts can be fixed. Thus, as one can always know the exact day of pregnancy of any doe, and, as the duration of pregnancy (namely 31-32 days) is fairly regular in any one strain of rabbits, the experiments can be repeated under almost identical conditions, on any particular day of pregnancy.

It was considered best to start with does at the end of pregnancy, to continue by working backwards in stages of pregnancy day by day, and to conclude on does at the very beginning of pregnancy. The reason for so doing was to ascertain, first of all, the minimum quantity of pituitary strong enough to induce parturition on the last day of gestation. In the case of the cat, already referred to, it was found that a dose of pituitary extract equal to 0.004 mgrm of moist posterior lobe per kilo cat is the

minimum that produces a visible effect on the uterus *in situ* This minimum amount was therefore taken as the starting-point The extract used was that of Parke, Davis and Co, which the author has recently standardised after the method suggested by Burn and Dale⁽⁹⁾, and has estimated to be equal to 30 mgrm of moist gland per 1 c c

The results obtained may be divided into three groups, namely, those concerning the time (1) from the 32nd backwards to the 29th day of pregnancy, (2) from the 28th to the 18th day of pregnancy, and (3) from the 17th to the 1st day of pregnancy They will now be described in the order in which they were actually worked out

*Group 1 Covering the time from the 32nd backwards
to the 29th day of pregnancy*

That the does were not under actual labour at the time when the injections were commenced was taken for granted because of the fact that they had not yet made a fur-nest, as they normally do some short time before they produce their young Furthermore, to ensure that the injection itself was not the cause of the onset of labour, from mechanical or other causes, control injections with saline solution were given without causing any visible effect Thus, it was made certain that all the occurrences following the pituitary injections were really the result of the drug administered In all the cases of this group immediate delivery of live foetuses could be induced by injecting a certain quantity of pituitary

The facts concerning the 32nd and 31st days of pregnancy will first be dealt with, as these results are more or less equivalent to each other, owing probably to slight variations in the length of pregnancy The smallest quantity of pituitary needed to cause immediate parturition was equal to 0.0075 mgrm of moist posterior lobe per kilo rabbit As a rule, the does showed distinct signs of straining immediately after the administration of the pituitary, and cast the first of their young within 1-3 minutes afterwards The action of this very small quantity of pituitary was just sufficient to give the uterus power to get rid of one foetus, and no more When, after an interval of 4-6 hours, a second injection of the same minute dose of pituitary was administered, the second foetus was born and so on, showing that the pituitary action was so slight and short in duration that it was exhausted with the delivery of a single foetus When the dose of pituitary was increased to an amount 10-20 times as large as the minimum quantity a different thing happened, namely, one foetus after another was cast, so that within 5-10 minutes

the whole litter was born. At a normal birth the young rabbit is born enclosed in the foetal membranes and placenta, so that the whole ovum is cast *in toto*, under the action of pituitary the young, especially the first one, were very often born without the placenta, which usually followed a short time afterwards, sometimes as a result of a second injection.

The results obtained on the 30th day of pregnancy did not differ in nature from those described above, but for inducing parturition on this day a much larger quantity of pituitary was required than on the last two days of pregnancy, namely, a dose equal to 0.3 mgrm of moist gland. With the increase of the dose of pituitary there was an obvious delay in the response, as the actual birth occurred usually only after prolonged treatment extending over several hours.

On the 29th day of pregnancy a further increase of pituitary was needed in order to obtain the same result as on the last three days of gestation. The minimum quantity was found to be equal to 0.6 mgrm of moist gland per kilo rabbit and the number of injections required to produce birth varied between one and eight. On this day in particular it very often happened that the response of the uterus to the pituitary injection was the delivery of one young only, for example, one doe was given three injections of pituitary at hourly intervals, 5 minutes after the third injection one foetus, looking very underdeveloped, yet alive, was cast without the placenta. No more injections were given on this day, but three days afterwards, on the 32nd day of pregnancy (the normal duration), another nine fully developed young were born, these averaging 20 gm heavier than the one produced by the action of pituitary on the 29th day.

In short, it was possible to induce parturition by injecting a certain quantity of pituitary on the 32nd, 31st, 30th and 29th day of pregnancy. The minimum dose of pituitary strong enough to cause delivery on the 32nd and 31st day appeared to be equal to 0.0075 mgrm of moist gland per kilo rabbit, rising to 0.3 mgrm on the 30th day, and to 0.6 mgrm on the 29th day of pregnancy. The complete delivery of the litter could always be induced, and this depended only on the amount of pituitary injected.

Group 2. Covering the time from the 28th to the 18th day of pregnancy

In all experiments of this group the does were given nine injections of pituitary at hourly intervals, so that they were kept under continual pituitary action throughout 9 hours. It may be said at once that this

period is characterised by the fact that no quantity of pituitary was ever found sufficient to induce abortion during the day on which the experiment was carried out. As a result of this prolonged pituitary action, however, the foetuses were killed inside the uterus, and were cast some days subsequently. After this fact had been established, the next step was to find out approximately the minimum quantity of pituitary sufficient to cause the death of the foetuses on the various days of this period. It was found that a dose of pituitary extract equal to 1.3 mgrm of moist gland per kilo rabbit administered for every injection on the 28th day of pregnancy, a dose equal to 1.5 mgrm of moist gland administered on the 27th day, and a dose equal to 2 mgrm of moist gland given on all the other days back to the 18th day of pregnancy, was succeeded by abortion some days subsequently. The interval between the injections and the subsequent abortion varied from 2-6 days. In some cases, in which the experiment was performed on the 26th and 27th days of pregnancy, abortion occurred at the normal end of pregnancy, namely on the 32nd day. That the foetuses had died in these cases in consequence of the pituitary action seemed to be proved by their appearance, in that they were distinctly underdeveloped, the stage of development varying with the day of pregnancy on which the injections had been made, and were far lighter in weight than the average young born normally. In some cases, however, one or two young escaped death and were born alive amidst the emaciated mass of the dead litter, while in other cases, where all the foetuses were dead, it appeared as if the foetuses had died off at different times, for they varied in their degree of development and in their stage of decomposition.

To elucidate the actual cause of foetal death some does were killed after the injections had been made and before abortion occurred. For this purpose a group of four does was treated in the usual way on the 20th day of pregnancy. One of these was killed on the following day, or 18 hours after the last injection had been given. Reddish brown (decomposed) blood was found in the vagina, obviously having flowed out from the uterine cavity. The amniotic cavities were filled with a bloody fluid, and all the foetuses had been killed, and were already showing distinct signs of decomposition. The placentas showed a pronounced stage of destruction, and were dark blue in colour, this presumably being caused by acute bleeding into their tissues.

Another doe of this group was killed on the second day after the injections, that is on the 22nd day of pregnancy, and it was observed that some of the foetuses lying in the ovarian ends of both the uterine

horns were still alive, but that the majority of the young had been killed and were in an advanced degree of autolysis

The third doe, killed three days after the experiment, on the 23rd day of pregnancy, presented a similar picture of destruction and atrophy inside the uterine horns, but still further advanced than in the former cases

The last doe, killed four days after the pituitary treatment, on the 24th day of pregnancy, had already aborted and showed only the usual symptoms of recent pregnancy

These experiments were repeated and gave exactly the same results as before. There again it was found that in one case, which was treated with pituitary on its 21st day of pregnancy and killed three days subsequently, both foetuses occupying the ovarian ends of the two uterine horns had survived, whilst all the others had been killed

Although these experiments had given evidence of foetal death following pituitary action, they had yielded no clear indication of when death actually occurs, nor of what might be regarded as the primary cause of death. In pursuit of this question another group of four does was selected and treated in the usual way on their 21st day of pregnancy

Two (*a* and *b*) of these were destroyed one hour and a half after the last injection of pituitary had been given. It was surprising to find in one of them (*a*) that the foetuses were still alive, although apparently injured, as they showed a distinct lack of activity. The placentas, however, looked slightly congested and were a shade darker in colour than usual

In the second case (*b*), the foetuses and placentas showed a degree of destruction distinctly more pronounced than in the other doe, the foetuses were dead, and the placentas congested. In both these cases, however, no free blood could be observed, either in the vagina or anywhere in the uterus

The third doe (*c*) of this group was killed 5 hours after the last injection had been administered. In this case the effect of pituitary action was obviously more marked than in the other two does. The advanced atrophy of most of the foetuses showed that they must have died some hours previously. Only the two lying at the ovarian ends of the uterine horns had escaped death up to this time, but they were distinctly inactive

In the fourth animal (*d*) of this group, which was killed 10 hours after the last pituitary administration, the degeneration of the foetuses and placentas was more advanced than in the other three

These experiments showed that the immediate effect of pituitary action on rabbits in the 20th or 21st day of pregnancy was small, presumably causing some disturbance in the foetal circulation, followed by a slow dying of the foetuses. It then became of interest to determine whether the same effect was produced by an equal quantity of pituitary extract given to animals in a more advanced stage of pregnancy. For this reason three rabbits were taken, one on the 24th day, the second on the 26th day, and the third on the 28th day of pregnancy, all three were treated exactly in the same way, and were killed 3 hours after the last injection had been administered.

An examination of the organs in these three rabbits showed an astounding amount of destruction increasing with the advance in the stage of pregnancy. In the case on the 24th day of pregnancy the foetuses were dead and the placenta congested, the degree of disturbance was greater in the second rabbit, and in the third the foetuses already showed an advanced stage of autolysis, the placentas were black and blood had flowed into the liquor amni and into the vagina, indicating that the action following the first few injections had been strong enough to kill the foetuses.

The actual cause of the death of the foetuses in the experiments of this group was found to be a flow of blood into the tissue of the spongy layer of the placenta. The degree of acute bleeding as an immediate result of pituitary action varied according to the stage of pregnancy at which the animal happened to be. In the earlier stages of pregnancy, as on the 20th day, the effect on the placenta could scarcely be detected by the naked eye when the doe was killed a few hours after the last pituitary injection had been given, though histological preparations showed that some tiny amount of bleeding had occurred as a result of the abnormal tearing of the uterine muscle at the joint layer of the placenta. But as soon as bleeding, however minute in quantity, took place, there was sufficient cause to make bleeding continue indefinitely until large blood-clots were formed, which worked like wedges between the uterine wall and the placenta and finally led to the complete detachment of these two organs. The further pregnancy had advanced the greater was the immediate effect of pituitary action, represented by coagulated blood of increasing amount lying in the meshes of the destroyed tissue of the spongy layer.

Attention may be drawn to the fact that in a number of cases the foetuses at the vaginal end of the uterine cavity had been killed, whilst those at the top were still alive, as though the pituitary extract had

acted differently upon each part of the uterus. The explanation of this, however, might be that the bottom foetus has to stand the pressure from all the others lying further up, whilst the one at the top has to bear only the contractions of the surrounding uterine wall. Besides, it may be due also to the way in which the uterus is fixed in position, for the vaginal part of it is certainly more firmly attached to its surroundings than the ovarian part, and thus, the uterus contracting into a fixed point at the bottom, the effects of labour are concentrated at this particular spot. To sum up, it may be said that the effect of pituitary action upon the pregnant uterus during this period consists of some destruction of the tissue of the spongy layer, and that this increases considerably with the advance of pregnancy and causes a corresponding amount of bleeding, which finally brings about foetal death and abortion.

Group 3 Covering the time from the 17th to the 1st day of pregnancy

This group of experiments is characterised by the fact that whatever reasonable quantity of pituitary extract was injected into a rabbit in this period of pregnancy it was never possible to disturb pregnancy. The amount of pituitary used at each injection was up to 0.5 c.c. of pituitum (Parke, Davis), which is equal to 15 mgrm. of moist gland, or 4-5 mgrm. per kilo rabbit. Considering that the average weight of the posterior lobe of the hypophysis in the rabbit is 7.5 mgrm., the quantity of pituitary used for each injection was far in excess of any amount which could possibly be secreted naturally, and therefore was thought to be strong enough to produce a maximum action on the uterine muscle. That the action of such a dose of pituitary was really powerful could easily be observed by the distinct straining which the rabbit showed immediately after the injection, and the frequency of micturition and defaecation gave evidence of its violent action upon the plain muscle of other systems. Twenty-seven experiments were carried out on rabbits which were in the different days of this period of pregnancy, and it was found that no pituitary action could be produced sufficient to disturb pregnancy during this time nor sufficient even to reduce the size of the litters born.

Discussion

The results obtained in this investigation indicate that considerable changes occur in the relationship between the hypophysis and the uterine muscle during pregnancy, and it is now a matter of interpreting these changes rightly. They may be due to variations in the functions of

one of these organs or in both of them to decide this question it would appear best to start first with the study of the results in Group 1

It has been shown that it is possible to induce parturition on any day of this period by stimulating the uterine muscle with a certain quantity of pituitary. But in order to obtain always the same result it is necessary to increase the dose of pituitary from a quantity equal to 0.0075 mgrm of moist gland on the 32nd and 31st day of pregnancy to one equal to 0.3 mgrm on the 30th day, and to one equal to 0.6 mgrm on the 29th day of pregnancy. This looks as though the conditions in the uterine muscle were more or less the same all the time, and as though there were naturally a rapid rise of pituitary secretion finally resulting in labour. As the average weight of the posterior lobe of the pituitary gland in the rabbit is 7.5 mgrm, it seems quite likely that this gland is able to secrete $1/1000$ of its total weight per hour, which amount is required to cause birth on the 32nd and 31st day of pregnancy. This sounds acceptable enough, but that there should be an increase of secretion at a rate of $1/100$, roughly speaking, within two days, is not so easy to believe.

This figure is arrived at as follows. It may be assumed that the uterine muscle does not change in its functions from the 29th day up to the 31st or 32nd day of pregnancy, because it is capable of delivering the young at any time during this period, provided it gets the requisite stimulus from the pituitary. Furthermore, let us suppose that a negligible quantity of pituitary is secreted on the 29th day of pregnancy, and that a dose of pituitary equal to 0.6 mgrm of moist gland is therefore needed to induce parturition. As roughly $1/100$ of that amount only is required to yield the same effect on the 31st or 32nd day of pregnancy, we could say that the other $99/100$ of that amount is naturally secreted on these two days, thus stimulating the uterine muscle to such an extent that we have only to add the minute quantity of 0.0075 mgrm to make the uterus reach the point where it gets rid of its contents. This would mean, in other words, that the pituitary gland would secrete an amount equal to the difference between 0.6 and 0.0075 mgrm of moist gland per hour on the 31st and 32nd day, or an amount approximately equal to its own weight per day. This would be a considerable but not an impossible quantity. From this argument the conclusion might be drawn that labour is brought about by a sudden increase of pituitary secretion during the last days of pregnancy. If this be a true conclusion, we ought to be able to obtain some confirmation of its truth by studying the results of the second group of experiments.

Coming now to Group 2 of the experiments, we find, however, that

there is a sudden break in the course of events occurring between the 29th day and the 28th day of pregnancy. We find that when the dose is confined within reasonable limits, pituitary injected into a rabbit on the 28th day of pregnancy will never directly cause abortion. The fact that pregnancy can be interrupted by injecting pituitary on the 29th day, and cannot be interrupted by doing so on the 28th day, in spite of using a quantity of pituitary far larger than could ever possibly be secreted by the hypophysis of the rabbit, proves that there must be something besides the pituitary secretion that changes in its function during pregnancy, and this can only be the uterine muscle. The conclusion is that the abortion caused by the administration of pituitary on the 29th day of pregnancy cannot possibly be due to an increase of pituitary secretion in the rabbit, but must depend on some notable change in the uterine muscle occurring at that particular time.

Furthermore, let us consider the immediate effect of pituitary action upon uteri in different stages of pregnancy within this period. Let us suppose that during the time from the 18th to the 28th day of pregnancy the pituitary secretion is more or less steady, as Dixon and Marshall have shown, then one would expect to obtain more or less the same result on any day of this period if an equal quantity of pituitary is always used, but this is not so. It is beyond all doubt that the effect gradually increases when the experiments are carried out in the way described, and that these show only a minute effect at the beginning of the period but end, however, in vast destruction on the 28th day of pregnancy. This is a further proof that the uterine muscle undergoes a remarkable change, at least from the 18th day up to the 29th day of pregnancy.

Lastly, when we follow the results described in Group 3 of the experiments we experience yet another surprise, for we find that the pituitary loses all its power to disturb pregnancy during the first 17 days of gestation. Here again, the remarkable break occurring from the 17th day to the 18th day of pregnancy can be explained only by some cause affecting the uterine muscle. We may be quite certain that we shall obtain maximum contractions of the uterus, lasting at least an hour, when 0.5 c.c. of pituitrin (Parke, Davis) is given to a rabbit. In spite, however, of extending this maximum action over 9 hours, it never results in disturbing pregnancy on any day of this period. Moreover, it does not produce even the slightest influence on the transport of the ova during the earlier days of pregnancy. In view of all these facts, therefore, we concluded that a great change takes place in the uterine muscle, beginning at least on the 17th day and gradually developing

up to the 29th day of pregnancy This period, in which the change in the uterine muscle is experimentally proved, covers 13 days of pregnancy, but it remains an open question whether the change commences earlier than we are able to recognise it and continues to develop during the last few days of pregnancy or not In order to solve this question we may consider the evidence supplied by other investigations on the uterine muscle during pregnancy

It is universally recognised that the increase in weight of the uterus during pregnancy is chiefly due to the hypertrophy of the muscle Luschka and Veit(3) found in the human uterus that the formation of new muscle fibres is limited to the first three or four months and that from this time onwards only hypertrophy of the existing muscle cells takes place, each cell increasing 7-11 times in length and 2-7 times in width A similar increase in the size of the uterine muscle during pregnancy may be postulated also of most mammals

In Fig 1 is shown a chart kindly given to me by Mr J Hammond,

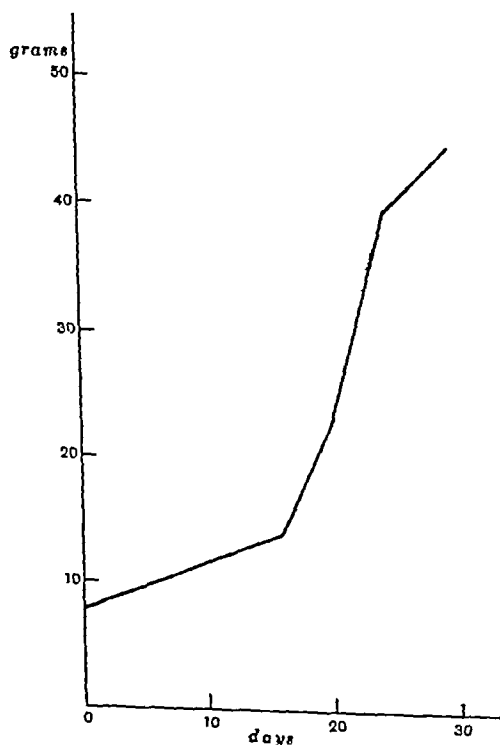


Fig 1 The rate of growth of the uterine muscle of the rabbit during pregnancy

which shows the rate of growth of the rabbit's uterus during pregnancy. It gives a clear idea of the slight increase in weight during the first 16 days of pregnancy, and of the rapid growth of the uterus that takes place from this time onwards.

We know very little about the physiology of the uterine muscle during pregnancy, but clinical observations throw some light upon the remarkable change which occurs during its progress. We may quote a statement on this matter from Williams' *Obstetrics* (4) "The increasing readiness with which the uterus reacts to stimulation during the latter months of pregnancy affords abundant evidence of its growing irritability. The intermittent contractions, which occur at intervals throughout pregnancy, come more and more frequently at this time, and occasionally with such intensity that it may be difficult in the last few weeks before delivery to distinguish between them and actual labour pains." This is the point of view expressed in most of the text-books on obstetrics, and adopted in physiology.

CONCLUSIONS

My own interpretation of these facts is as follows. There is no increase of irritability or sensitivity of the uterus during pregnancy, but, corresponding to the growth of each muscle cell, there is a regular rise of contractility of the muscle. The larger the muscle cell the greater is its ability to shorten itself. It is in consequence of this fact that the effect of pituitary extract upon the muscle increases steadily the further pregnancy is advanced. During the first ten days of pregnancy the maximum contraction in each muscle fibre cannot produce a sufficiently great mechanical effect to influence the transport of ova, or to disturb the connection between the uterine wall and the placenta during the subsequent seven days. In other words, at this time the uterine muscle cell has not yet grown enough, as may be seen from the chart (Fig 1), and so has not yet the power to break down the tissues of the spongy layer of the placenta when it is stimulated by pituitary. The maximum shortening of the cells caused by pituitary on the 18th day, however, has just reached the threshold where the attachment of the placenta begins to suffer, and from this time onwards there is a perceptible increase of effect with every day, the maximum contractions of the muscle following the pituitary administration causing vast destruction between foetal placenta and uterine wall. The transition of the pituitary effect from the 28th to the 29th day of pregnancy is by no means abrupt, as it might appear, on the contrary, when we look at the enormous devasta-

tion effected by pituitary action on the former day we wonder why actual delivery has not taken place. On the 29th day of pregnancy the uterine muscle cells have grown so large that their maximum contraction, caused by pituitary, results in the delivery of young, while in the last few days of pregnancy the uterus keeps on growing and increasing its contractility so that eventually a maximum contraction is no longer needed to produce the young. It has been shown above that there is a difference in the effect following a minimum dose of pituitary and that following a maximum dose given, for example, on the last day of pregnancy. The minimum dose of pituitary at this time may cause the birth of one young, but a larger dose is immediately followed by the birth of the whole litter. By the end of pregnancy, however, the muscle fibres have become enlarged to such an extent and have thus acquired so great a power of contractility that their spontaneous contractions alone finally cause birth. There is no need to postulate any sudden influence or stimulus acting on the uterus at this time, as the labour pains are not essentially different in character from the contractions immediately preceding them, but only slightly more severe in degree, and so are to be regarded as the last step in the series of gradually expanding events.

There are other factors which have been regarded as of importance in the onset of labour and therefore need some consideration. For example, it is said that the loosening of the placenta is indispensable to the delivery of the foetus. That this is not so can easily be proved. Under normal conditions young rabbits are cast wrapped in the foetal membranes, that is, the ovum is cast as a whole, but it has been shown above that the young can be cast without their membranes and placentas when pituitary is used, particularly so on the 29th and 30th day of pregnancy. This proves that the actual process of birth is merely a matter of the uterine muscle. In man and many mammals the foetus is born first and the placenta follows some time subsequently. Furthermore, it is well known that the placenta may not get loose at all, whilst the delivery of the foetus takes place quite normally. The conclusion to be drawn is, however, that the actual mechanism loosening the placenta as pregnancy advances is due, apart from any retrogressive processes in the placenta itself, to the increasing contractility of the uterine muscle. The placenta is a non-contractile body attached to the uterine wall, which, on the other hand, is continually contracting and extending. So long as the variation in the surface of the uterine muscle is comparatively small, a fixed attachment between these two organs is likely to exist. This is shown in the rabbit by the fact that up to about the 20th

day of pregnancy the placenta can only be separated from the uterine wall by cutting through the close connection between them. As pregnancy proceeds, however, this connection becomes looser and looser every day, so that at the end of gestation the placenta may be squeezed off from the uterine wall with the greatest ease and this loosening would certainly be brought about by the increased activity of the uterine muscle.

It has also been suggested that the size of the foetus and the simultaneous extension of the uterine wall must be looked upon as important factors in starting parturition. But attention must be called to the fact that in man the duration of pregnancy remains constant in spite of great variations in the size and weight of the foetus, and in spite of the enormous extension the uterus has to undergo in case of twins or hydramnios. Further, in cases of extra-uterine foetation, where the foetus has had a chance of growing up to the normal end of pregnancy, patients feel exactly the same pains at the onset of labour as under normal conditions, and if the placenta happens to be attached to the outside of the uterus it becomes loose in the same way as under ordinary conditions.

In this connection an interesting paper of Sauerbruch and Heyde(5) may be referred to. Using the method of parabiosis they tried to detect whether birth occurring in one of two partners influences the pregnancy of the other. Five experiments were carried out on rats, each rat of a pair being at a different stage of pregnancy. In two experiments the animals lived together for a week when one in each pair bore a normal litter without affecting their partners, which cast their young a fortnight later at their normal end of pregnancy. In the other three experiments the rats lived together longer, and the difference in the stage of pregnancy was not so great. Yet, in spite of the difference in the stage of pregnancy, labour started almost simultaneously in both animals and resulted in a litter of normal young in one, and an abortion of under-developed foetuses in the other partner. The authors conclude that the lack of mutual influence between the two partners observed in the former experiments was due to want of sensitivity of the uterine muscle in the partners that were at the beginning of pregnancy.

In another investigation on the induction of labour by injections of foetal serum Heyde(6) again comments on the lack of sensitiveness of the uterus at the earlier stages of pregnancy.

When we consider further how much effort has been expended, both legally as well as illegally, in attempting to discover a drug that will excite the uterus sufficiently to induce abortion, and how all this effort

has been in vain, we may say again that this failure is due to lack of sensitivity of the uterine muscle at the time

Lastly, we have to consider on what the growth of the uterine muscle during pregnancy depends. It can be at any rate stated that its growth is due to a hormone influence and not to anything else. So far as our present knowledge goes, we are entitled to regard the presence of the corpus luteum as the main factor in causing the growth of the uterine muscle, and this is particularly clearly illustrated in pseudo-pregnancy.

Hammond(8) has shown that in the rabbit the removal of the ovaries at any time during pregnancy is followed either by absorption of the foetuses or by abortion, this being almost certainly due to the disappearance of the corpora lutea. The reason why pregnancy ceases to go on under these circumstances is that the uterus immediately shrinks and atrophies. These facts and the results obtained in the present investigation furnish ample evidence that abortion occurring at earlier stages of pregnancy cannot be regarded as the effect of any activity of the uterine muscle, but rather as the consequence of some disturbance in the supply of the necessary hormones.

The author expresses his sincere thanks to Dr F. H. A. Marshall, and to Mr J. Hammond, for the great interest shown and the helpful criticism offered at all stages of this investigation.

The author's thanks are due to Messrs Parke, Davis and Co. for the free supply of the drug.

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ON CELLULAR ACTIVITY AND CELLULAR STRUCTURE AS STUDIED IN THE THYROID GLAND

BY W CRAMER AND R J LUDFORD

(From the Laboratories of the Imperial Cancer Research Fund)

THE following observations were made as an attempt to establish a correlation between changes in the functional activity and in the structure of the cytoplasm as indicated by alterations in the mitochondria and the Golgi apparatus. Both these structures can be seen in the living cell and are not, therefore, artefacts produced by special methods of fixation. The mitochondria have, moreover, been seen to undergo spontaneous changes in size and shape in living cells⁽¹⁾. We have used the cells of the thyroid gland as the most suitable test object for the following reasons. They are cells of a secreting gland, but unlike most gland cells they do not contain during rest their specific secretory product within themselves to any great extent. Most cells of a secreting gland are filled with their specific secretion—we may give as instances the acinar cells of the pancreas, the medullary cells of the adrenal, and the cells of the salivary glands, while the liver cells contain glycogen as their specific secretion. It is obvious that in such cells changes in the internal structure may result for purely mechanical reasons, owing to the accumulation, and subsequent rapid discharge of secretion in the course of functional activity. In the resting thyroid gland the specific secretion accumulates as the “colloid” outside the cells, within the lumen of an alveolus.

Another reason for selecting the thyroid gland was that previous observations by one of us⁽²⁾ had determined a number of conditions which are associated with rest and activity respectively of the gland. Thus, exposure to cold and the injection of β -tetrahydronaphthylamin produce an intense activity of the gland, while a resting condition is induced by exposure to a hot environment, and by thyroid feeding. The increased activity of the gland on exposure to cold and after injection of tetrahydronaphthylamin manifests itself by an intense congestion of the interalveolar and the intra-alveolar capillaries and by a disappearance of the colloid from the alveoli. A figure illustrating this condition

has been published in a previous paper(3) The congestion of the intra-alveolar capillaries may be so intense, especially after tetrahydronaphthylamin, that hæmorrhages into the centre of the alveoli may occur Sometimes the cells lining the alveoli get detached and are pushed into the central lumen of the alveoli Conversely heat and thyroid feeding induce inactivity of the gland We have then an accumulation of colloid in the alveoli which appear distended with colloid The interalveolar capillaries close up altogether so that the lining cells appear to be resting directly upon the basement membrane The thyroid gland offers, in fact, a very striking illustration of the opening and closing of capillaries in the different stages of functional activity of an organ The staining reaction of the colloid also changes In material fixed in Formol bichromate solution and treated subsequently with osmic acid (Schridde's method) the colloid of the resting thyroid gland, that is to say, the gland of a rat or mouse kept in a hot environment or fed on thyroid gland, stains deeply with Heidenhain's hæmatoxylin and retains the stain tenaciously on differentiation with iron-alum In the active gland the first change is the loss of this affinity for hæmatoxylin, so that the colloid appears colourless on differentiation The response of the thyroid to heat which was first described by one of us in 1916, has since been confirmed by Mills(4)

In a previous publication(3) has been discussed the significance which these observations have in demonstrating that the thyroid forms together with the adrenal a humoral mechanism for the heat regulation of the body In the present paper, where we are dealing with changes in the activity of the thyroid induced by different agencies we may point out the importance of controlling the thermal environment if one wishes to get comparable results It is probable that the neglect of this factor is responsible for many of the contradictory or inconclusive results recorded in the literature concerning changes in the appearance of the thyroid gland

As an illustration of the rapidity with which the thyroid responds to changes in the thermal environment, Figs 1, 2 and 3 are given here Fig 1 represents the thyroid of a mouse kept at ordinary room temperature Figs 2 and 3 give the appearance of the thyroids of mice which for a week had been subjected to the following routine They were kept in glass jars which were placed in an incubator kept at 37° C at 10 o'clock in the morning and removed at 5 o'clock in the evening From 5 p m till 10 a m of the following day they were kept in a room with an open window during cool weather in March After having been sub-

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jected to this regular routine for several days one mouse was killed in the afternoon after having been kept for 6 hours in the incubator. The



Fig 1 Room temperature



Fig 11 Immediate effect of cold, following heat

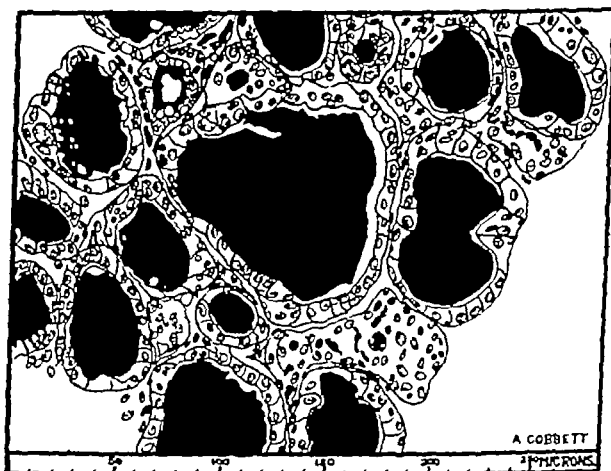


Fig 111 Immediate effect of heat, following cold

Figs 1-111 Thyroid of mouse $\times 300$

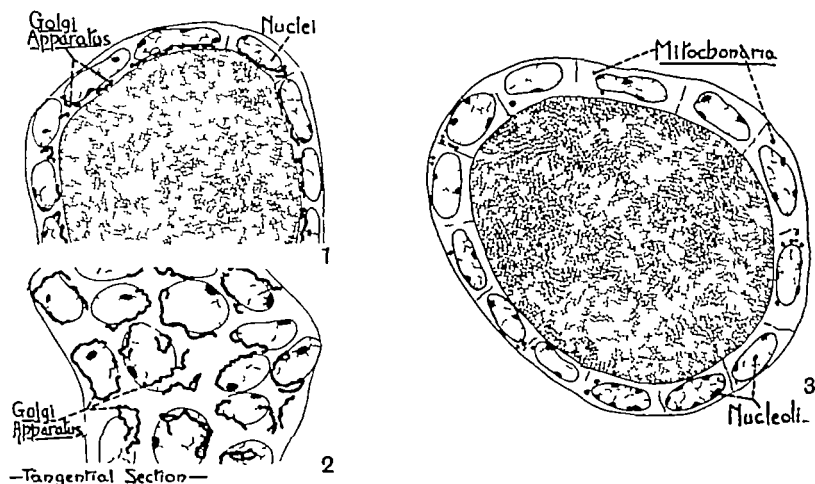
appearance of its thyroid which represents the immediate effect of a hot environment is given in Fig 111. The other mouse had been removed from the incubator at room temperature and kept in the cool room for 4 hours, when it was killed. It shows the immediate effect on the thyroid gland

of a cool environment as seen in Fig 11. This experiment has been repeated frequently with different variations, such as one single prolonged exposure to heat, with the uniform result that exposure to heat leads to an accumulation of deeply staining colloid, which on exposure even to a moderately cool environment rapidly disappears.

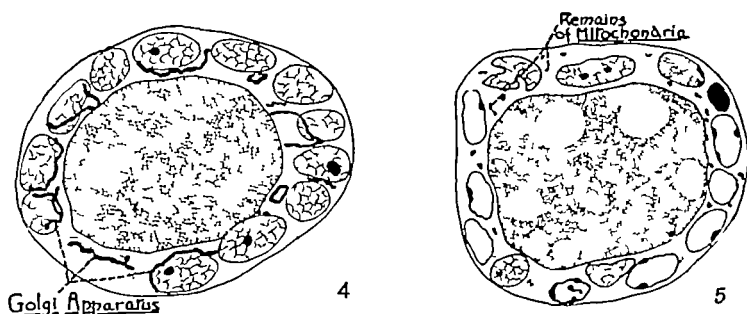
Having established a number of conditions which induce rapidly and with certainty rest and activity of the thyroid gland in such animals as the mouse and the rat, it was possible to study changes in cellular structure in these different conditions as manifested by changes in the mitochondria and in the Golgi apparatus in order to see whether cellular activity in the cells of the thyroid is correlated with definite changes in these cytoplasmic elements. So far little definite knowledge is available concerning the function of the mitochondria, although this problem has been the subject of a good deal of speculation. The mitochondria of the thyroid gland of guinea-pigs subjected to a number of different conditions have been studied by Nicholson⁽⁵⁾. His findings have, however, not given any very clear or conclusive results. Goetsch⁽⁶⁾ observed an increase in the number and size of the mitochondria in toxic adenomata of the thyroid associated with symptoms of hyperactivity of the gland. He pointed out the importance of finding a criterion for determining whether individual thyroid cells are functionally hyperactive or not, since many clinical cases of hyperthyroidism can frequently not be correlated with the ordinary histological appearance. He suggested that a study of the mitochondria might furnish such information. Quite recently Seecoff⁽⁷⁾ has published observations showing an increase in the mitochondria in the thyroid gland of guinea-pigs and rats in which hyperplasia had been induced by fat feeding and a diminution of the mitochondria when involution of these hyperplastic glands was induced by iodine. Here again we are dealing with chronic pathological conditions rather than a physiological response of the gland. No figures are presented recording these changes, which detracts from the value of these observations.

The Golgi apparatus has been studied by various authors in cells of externally secreting glands in different stages of secretory activity. In the resting cells it assumes a contracted and relatively simple form and is then closely applied to the nucleus with a definite orientation. During functional activity of the cells it has been found to undergo enlargement extending from the nucleus in the direction of the lumen. In many glands the secretion arises in intimate relationship with the apparatus, which later becomes broken up into fragments.

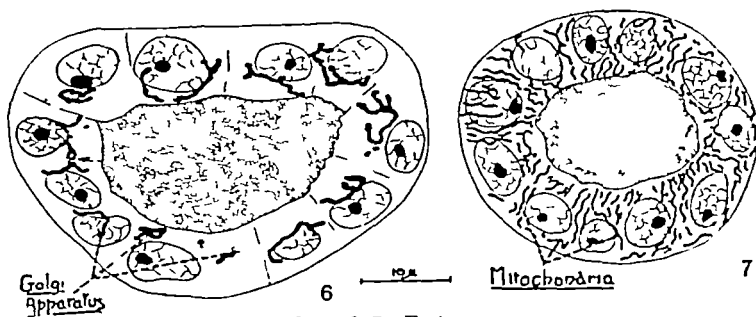
The methods employed by us for the demonstration of these two cytoplasmic structures were as follows For mitochondria Schridde's fixation (Formol bichromate solution with subsequent treatment by



Figs 1-3 Thyroid fed.

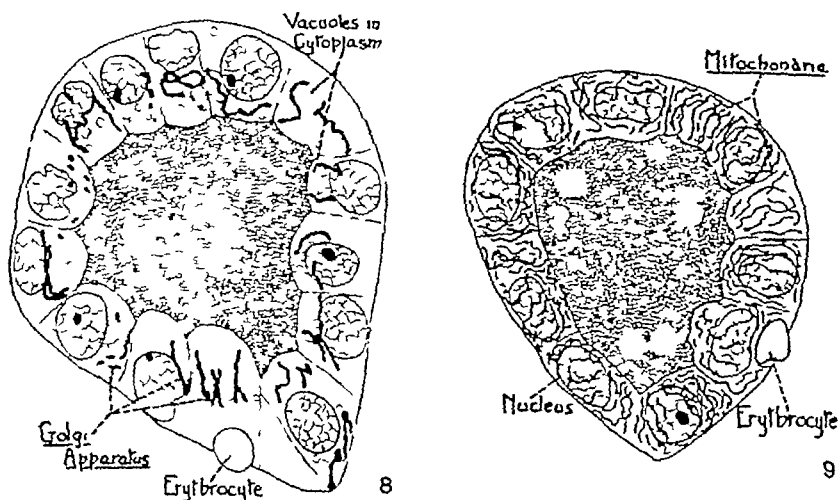


Figs 4, 5 Exposure to heat.



Figs 6, 7 Fasting

osmic acid) and staining with Heidenham's iron-alum hæmatoxylin. For the Golgi apparatus Ludford's(8) modification of the Mann-Kopsch method (fixation in osmic acid and sublimate and subsequent treatment with warm osmic acid) The actual appearances are given in Figs 1-11, to which the following details may be added Figs 1-3 from a rat fed on 0.1 grm thyroid daily for 30 days The animal was then in a good state of nutrition and the post-mortem examination showed no obvious changes from the normal. Fig 4 from a rat kept at 37° C for 3 hours Fig 5 from a rat kept at 37° C for 36 hours Fig 6 and Fig 7 from a rat kept without food for 20 hours in the animal room which was kept at a fairly even temperature of about 16-20° C This may be taken as representing a condition of normal activity of the thyroid gland in the rat Fig 8 from a rat which had been eplated and kept in the open air on a warm autumn day for 3 hours Fig 9 from a rat not eplated and

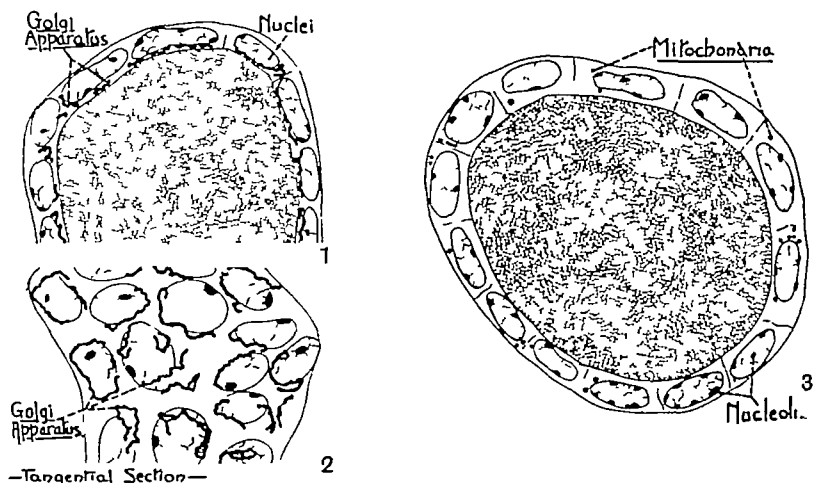


Figs 8, 9 Exposure to cold.

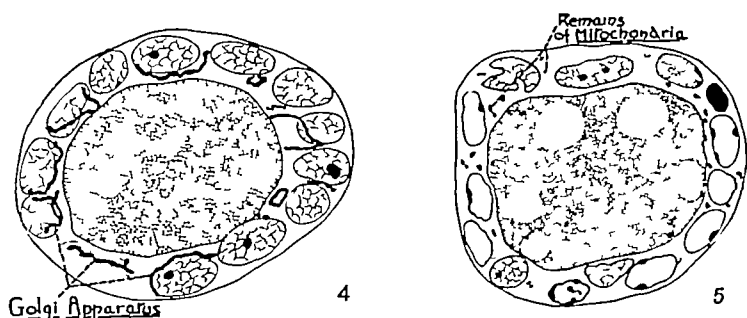
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The differences are particularly striking when one compares cells of

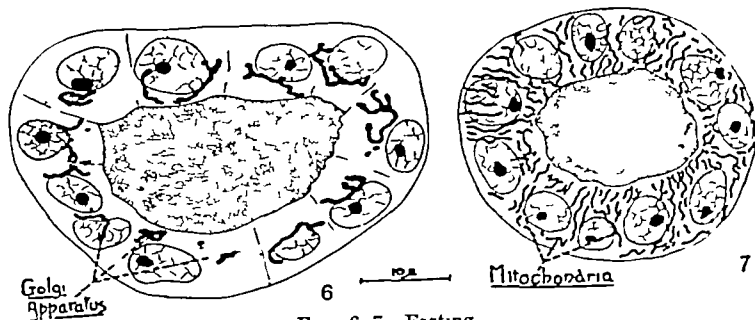
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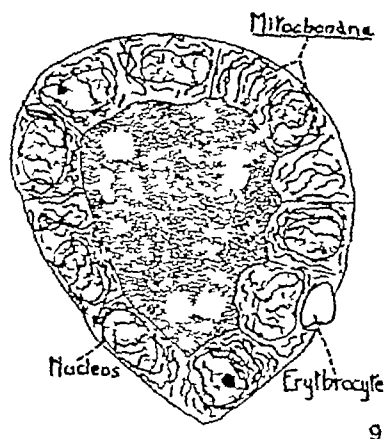
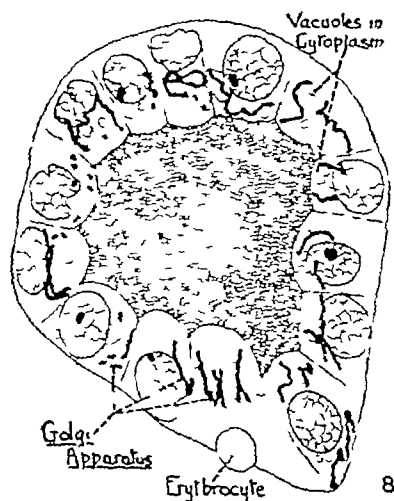


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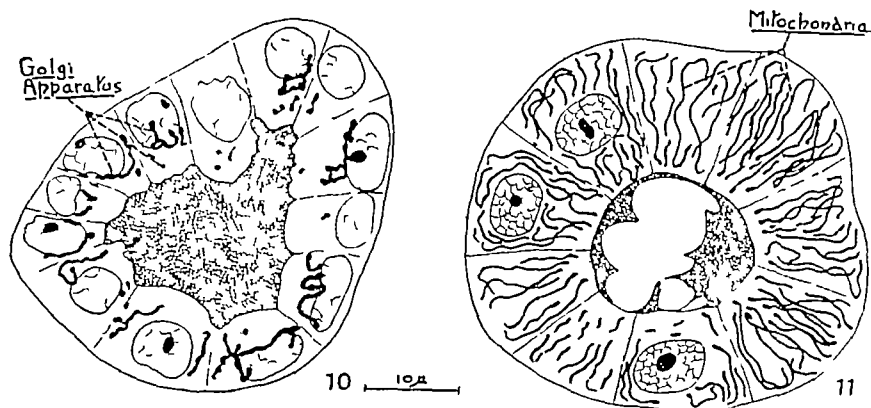


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The differences are particularly striking when one compares cells of

glands showing the two extreme stages, such as complete rest after exposure to heat and intense activity after injection of tetrahydronaph-



Figs 10, 11 Action of THN

thylamin In the cells of the thyroid of a normal animal all stages of activity may be seen in different alveoli, while the cells of each alveolus present a uniform appearance This is due to the fact that the cells of the thyroid function in relays, as do, in fact, the cells of all secreting glands In such glands as the pancreas or the adrenal medulla, where the specific secretion can be demonstrated within the cells, one finds that while the cells of a resting gland are fairly uniformly charged, they do not participate equally in the process of secretion when the gland is stimulated to activity In the active pancreas for instance one may find groups of acini having discharged completely their zymogen granules, while others are still filled with them Similarly, in the active adrenal medulla secretion begins always in the cells lying around the central vein and its immediate tributaries These cells will show profound changes, while in parts of the gland remote from this region the cells may still present an appearance of rest In the thyroid we find similarly a fairly homogeneous appearance in the cells of the completely resting gland while in the active gland different alveoli may present different stages of activity The figures represent the predominant appearance under the conditions indicated

The figures are self-explanatory, so that a detailed description is unnecessary A pictographic summary showing the changes in the mitochondria and the Golgi apparatus in a semi-diagrammatic form is given in Fig 12 The results may be summarised as showing a definite correlation between cell activity and cytoplasmic structure with increasing

activity the mitochondria become more and more differentiated from the cytoplasm, they enlarge and become filamentous, while with diminishing

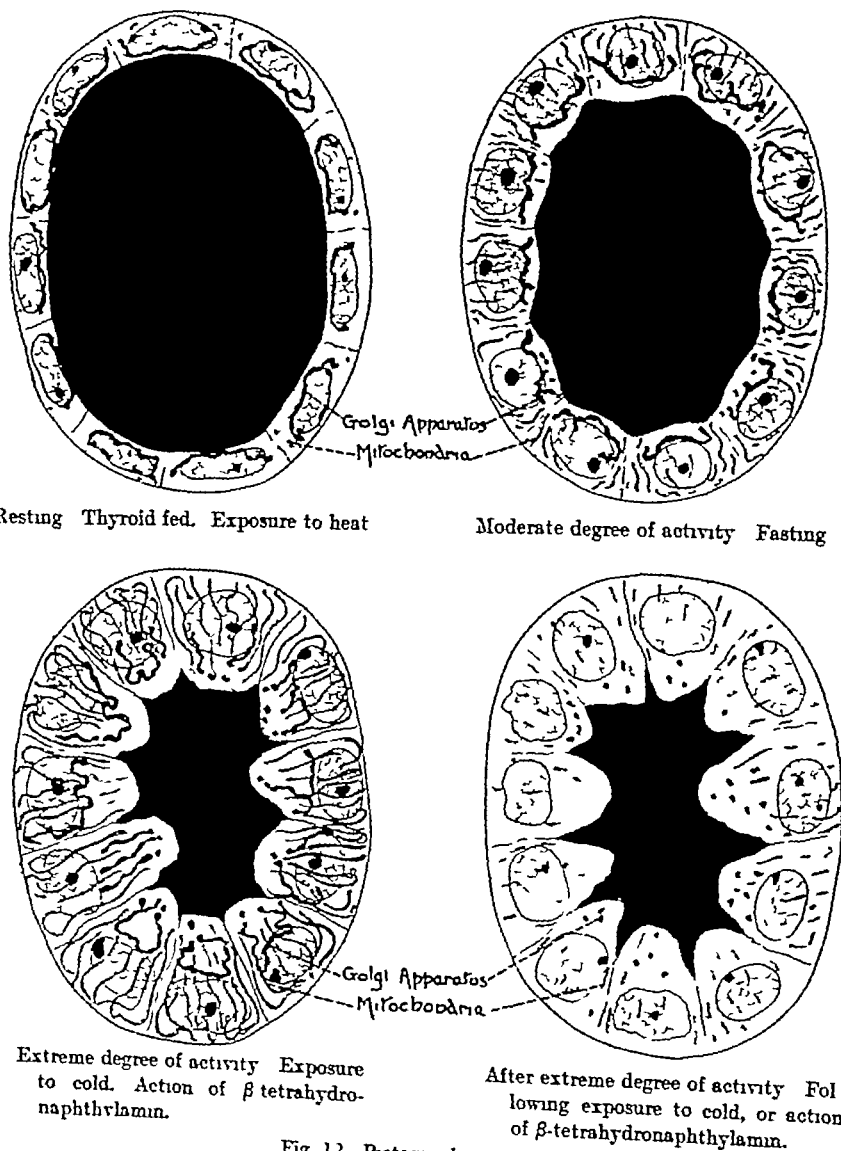


Fig 12 Pictographic summary

activity their differentiation from the cytoplasm becomes less distinct so that only a few granules remain visible After prolonged increased

activity of the gland the large thread-like forms break up into shorter rods

The Golgi apparatus shows the same changes which have been observed in cells of externally secreting glands in different stages of secretory activity, it exhibits a simple contracted form in the resting cell, during activity it enlarges, becomes convoluted, and after prolonged activity it breaks up. In the thyroid we find similarly the simple contracted form after thyroid feeding and after exposure to heat, while after exposure to cold and after injection of tetrahydronaphthylamin it is enlarged and convoluted and begins to disintegrate. This is itself confirmatory evidence that the various conditions examined by us induced rest and activity of the thyroid.

Cowdry⁽⁹⁾ has observed changes in the position of the Golgi apparatus in the thyroid gland of the guinea-pig and has suggested that this position might vary with the direction in which the specific hormone is secreted. He found that in some alveoli the Golgi apparatus had the usual orientation, lying on the side of the nucleus directed towards the alveolar lumen, while in others it was lying on the other side of the nucleus. He makes the interesting suggestion that in the resting gland, when the specific hormone is secreted into the central alveolar lumen, the Golgi apparatus lies on the side of the nucleus directed towards the centre of the alveolus. He assumes that during activity the thyroid hormone is secreted directly into the blood vessels or lymphatics and that the Golgi apparatus wanders round the nucleus with the change in the direction of secretion and comes to lie on the opposite side. This suggestion is of importance, because, if correct it would enable us to use the position of the Golgi apparatus in the thyroid cell as an indicator of its activity. There is, however, no evidence in Cowdry's observations of an increased activity of the gland, where he found that the Golgi apparatus had reversed its position. In our observations where the state of functional activity of the gland was known, we have found no evidence to support the suggestion that the Golgi apparatus exhibits a consistent change of polarity varying with activity of the gland, although occasionally it has been seen to lie on the side of the nucleus away from the centre of the alveolus. An example of this appearance is seen in one cell of Fig 8. Since in our preparations the position of the Golgi apparatus showed no consistent change in the conditions of extreme activity, it appears to us doubtful, in the absence of further evidence, whether Cowdry's interesting interpretation can be accepted.

In addition to the changes in the cytoplasm there are also nuclear

changes The most obvious change is a diminution of the chromatin content as evidenced by a loss of staining power of the nucleus in conditions of intense secretory activity

The findings recorded in this paper have a general interest from the point of view of cell mechanics In discussing the part played in the cell by surface energy due to structural surfaces account has been taken hitherto only of the so-called cell membrane which is really the most peripheral part of the cytoplasm, and the nuclear membrane The cytoplasm has been described erroneously as homogeneous and without visible structure by authors dealing with cell mechanics, Bayliss(10) and Leathes(11) for instance, though conclusive evidence to the contrary has been furnished by cytologists, as detailed in Cowdry's book In the mitochondrial apparatus the cytoplasm has a mechanism by which it can alter surface energy by creating within itself an enormous surface, or reducing the surface to a minimum. In the thyroid cells the former alternative is associated with intense activity, the latter with a condition of rest We have obtained evidence of a similar relationship in the adrenal medulla and the liver Now the concentration of lipoids in the cell membrane can be explained on physico-chemical grounds as being due to the fact that substances which lower surface tension tend to accumulate at the surface The same will occur therefore within the cytoplasm when a very extensive surface is created by the enlargement of the mitochondria The cytoplasmic lipoids will accumulate around the mitochondria It is interesting to note that on the basis of their staining reactions the mitochondria have been described as being composed of a core of protein surrounded by a sheath of lipoids and further that the formation of fat globules in cells has been associated with mitochondrial activity As the mitochondrial surface diminishes the lipoids will return to the cytoplasm This ebb and flow of the lipoids from the cytoplasm to the mitochondrial surface and back which accompanies changes in the mitochondrial surface, must necessarily affect the concentration of the lipoids in the cytoplasm and therefore also in the cell membrane With increased activity of the cell and increasing mitochondrial surface the lipoids will be withdrawn from the cell membrane to the mitochondria During rest the change will be in the opposite direction Such a process would account for alterations in the permeability of the cell

SUMMARY

Conditions were established which induce intense activity and complete inactivity respectively of the thyroid glands. A very clear relationship could be demonstrated between the functional state of the gland and the mitochondria. In the resting glands the mitochondria are barely visible. In the active gland they show an enormous enlargement. The condition of the mitochondria presents therefore a criterion for the functional state of the gland, and may, as Goetsch has suggested, be useful in the study of pathological conditions of the thyroid. The Golgi apparatus shows the same changes which have been observed in the other gland cells in rest and activity: a simple, contracted form in the resting cell, an enlarged convoluted form during activity, followed by a disintegration into granules.

The significance of the mitochondrial changes from the point of view of cell mechanics is discussed. The mitochondria represent a mechanism by which the cell can produce great variations in surface energy within the cytoplasm. On the basis of accepted views such variations must be accompanied by changes in the distribution of the lipoids within the cytoplasm and the cell membrane. These changes, in turn, would affect the permeability of the cell.

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THE VENOUS PRESSURE OF THE EYE AND ITS RELATION TO THE INTRA-OCULAR PRESSURE

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In the estimation of the venous pressure of the eye, five different methods have hitherto been employed with results so much at variance that the whole question of the physiology of the aqueous is still a matter of dispute. By some observers it is held that a pressure difference exists between the chambers of the eye and the exit veins such as to allow a drainage of fluid by a hydrostatic flow, by others that absorption occurs by osmotic forces alone, some have concluded that the pressure gradient from capillary to aqueous is sufficient to allow the formation of the latter by a process of simple transudation, while others insist that it is not sufficient, and that consequently the mechanism of formation is a secretory one, involving an active expenditure of energy by the cells of the ciliary body.

These experimental procedures may be briefly reviewed

1 The compression method of v Recklinghausen was employed by Seidel (29) and Hiroishi (14) who applying a pressure chamber over the episcleral veins as they are seen under the conjunctiva found the pressure at the point of their obliteration to be very much lower (10-18 mm. Hg in dogs and rabbits) than the intra ocular pressure. The measurement here made, however, is that of the pressure in the veins outside the eye—virtually in the orbit and to assume that it represents the intra ocular venous pressure is quite unjustified.

2 A very large number of experimenters, most recently Seidel (28) and Hiroishi (14), have drawn conclusions as to the method of exit of the intra-ocular fluids, and the relative pressures in the eye and in the veins, from experiments involving the injection of dyes. A dye having been introduced into the anterior chamber, the appearance of colouring matter in the episcleral veins is taken as necessarily indicating a fall in pressure between these two points. The introduction of a needle, however, into the eye, and the injection therinto of any material profoundly changes the pressure equilibrium, and completely alters the circulation. The conditions thus obtaining can in no way be called normal, and therefore physiological conclusions based on intra ocular injections are patently open to criticism.

3 Weiss (35), experimenting on rabbits, inserted a cannula into a vortex vein, thus measuring the pressure directly. He obtained very varying results. The venous pressure ranged from 33 to 63 mm. Hg and was invariably higher than the intra ocular, the ratio varying from 12 to 19. Lullies (19), using the same technique in dogs, came to the same conclusion his results varying from 21 to 39 mm. Hg. The blockage of a vortex

vein, however, involves the production of hyperæmia with which the collateral venous channels are unable to cope, and this invalidates any pressure readings thus obtained. The inadequacy of the anastomoses can readily be seen in the case of the albino rabbit if a ligature be passed round one of these veins subconjunctively, a zone of hyperæmia appears, which is seen most clearly in the iris, limited to the quadrant drained by the vein in question, later a considerable rise of intra-ocular pressure occurs (10–20 mm Hg). In the dog the same reaction occurs although it is much less pronounced, a rise in pressure of 7–10 mm resulting. The variation in rise of pressure is due to a difference in the richness in anastomoses in these animals, in the dog there is a direct communication between the anterior (ciliary) system of veins and the posterior (vortex), in the rabbit there is not, on obliterating one efferent channel in the dog, therefore, more anastomotic avenues are available than in the rabbit, and the rise of pressure is less marked. Lullies' results are consequently more nearly correct than those of Weiss, and of the former's the lowest come nearest to the truth.

4. With a view to determining the relative pressure relation between the aqueous and the venous channels, Wedgefarth (34) introduced a fine needle into an episcleral vein in the dog, pushed it down through the sclera into the anterior chamber, and then withdrew it and ligated the vein, since no blood flowed into the eye, he concluded that the pressure in the latter was greater than the venous pressure. A fistula was here made, however, at a moment when the normal pressure equilibrium was completely disturbed, it was made through raw tissue, and immediately afterwards the blood flow was stopped, thus providing every opportunity for the formation of clots, while the methods adopted subsequently to demonstrate the patency of the channel were merely such as would wash away any newly formed clot which might have been deposited at the time of fistulisation.

5. Bailliant (2) and Magitot (22) have made an extensive study of the ocular circulation by making use of a compression tonometer, which registers the force applied by the excursion of a standardised spring. On compressing the globe of the eye with the foot-piece of this instrument, the veins at the optic disc are simultaneously observed through the ophthalmoscope. In a certain proportion of eyes there is a spontaneous pulse in these veins just at their exit at the optic nerve head (58 p.c., Bailliant), the movement of pressure on the globe necessary to abolish this pulse shows how much higher the maximum venous pressure is than the intra-ocular pressure. Of the others which show no spontaneous pulse, 12 p.c. show the pulse if the globe is compressed, the pressure thus required to produce the pulse shows again how much higher the venous pressure is than the intra-ocular. In the remaining 30 p.c. no pulse can be elicited on pressure, in these the venous pressure is consistently below the intra-ocular. These deductions are, however, open to criticism, since, as will be pointed out later, several complicating factors other than mere pressure differences enter into the mechanics of the formation of the venous pulse of the eye.

The pressure in the intra-scleral veins. The consideration of the above results leads to the conclusion that the essential feature of any technique to determine the pressures of the venous exits must be the maintenance of a normal intra-ocular pressure as well as the avoidance of any venous hyperæmia. As best fulfilling these conditions, the technique employed by Carrier and Rehberg (7) in the estimation of capillary pressure was adopted and modified to suit the conditions of the case.

Dogs were employed in the investigation, since these are the most convenient laboratory animal with tolerably large veins, and in them,

as we have seen, the anastomoses of the venous channels are very efficient. Injection experiments in these animals show that the venous blood from the uveal tract is drained by two systems (a) from the anterior part of the choroid, the ciliary body, and iris by a complicated, inter-anastomosing ring plexus in the substance of the sclera (taking the place of the Canal of Schlemm) which empties into a second ring-plexus—the Circle of Hovius—running round behind the corneo-scleral junction, near the surface of the sclera, but still in its substance, being covered by a thin layer of scleral tissue through which it is clearly visible, (b) from the main body and posterior part of the choroid by four or five vortex veins which leave the eye equatorially and are carried away via the recti muscles. The Circle of Hovius is drained by two veins, or groups of veins, anteriorly, running forwards to join the orbital veins, and posteriorly by intra-scleral vessels, running backwards to anastomose with the vortices, lying, like the Circle of Hovius itself, in the outer layers of the sclera, and visible through its substance.

A piece of glass tubing 4 mm in diameter was drawn to a very fine sharp point at the end, the process being repeated several times to obtain a very short and fine tip. A side arm near the point was connected by a rubber tube to a levelling bulb filled with physiological citrate solution. As the levelling bulb is raised and lowered the citrate column in the tube follows suit, and a pressure is therefore exerted at the capillary tip equal to the height of this column. When the tip is immersed in blood, there is a theoretical error in the pressure measurements obtained depending on the difference between the surface tension of the citrate solution and blood, but its magnitude is so small that it may be neglected. When the tip is inserted into the lumen of a vein, blood will flow into the tube if the venous pressure is higher than the pressure of the citrate column, if the venous pressure is lower, citrate will flow into the vein. By raising and lowering the levelling bulb and observing the capillary point through a dissecting microscope, a very exact pressure measurement can be obtained. The intra-scleral veins of the Circle of Hovius and the posterior anastomosing veins just described are ideal for the method, since under the microscope they appear large enough for the capillary point to be introduced into them without hesitation and for it to be held in their lumen for some time. Meanwhile, the investing scleral tissue, by keeping the vessel patent, ensures the ready entrance of the point into the lumen, and at the same time allows perfect freedom for the continuance of the circulation (observable under the microscope), thus preventing any hyperæmia or damming up of the

stream The lateral pressure is thus measured while retaining a free and undisturbed flow in the veins, and at the same time leaving the intra-ocular conditions wholly unaffected The degree of accuracy attained is seen in that the mean variation of six readings taken in each experiment was ± 0.25 c.c. citrate (0.18 mm Hg)

Anæsthesia was induced by chloroform-ether, and maintained by intravenous chloralose The scleral veins were reached by slitting up the lids back to the orbital margin in the mid-line, and keeping them retroverted by stitches The conjunctiva was then incised behind the limbus, and dissected back until the veins were exposed The dissecting microscope was then focussed on the exposed sclera, direct illumination being obtained from a strong light concentrated by a lens system

Precautions were taken to record the intra-ocular pressure with as little disturbance as possible In the first experiment it was taken directly by a compensated manometer filled with saline which was inserted into the eye, using an air bubble inserted into a horizontal capillary tube as an index of equilibrium, and no measurements were taken until 15 minutes after the pressure changes following the introduction of the needle had disappeared In the second experiment the tension of the two eyes was taken by a tonometer and was found to be equal this instrument does not give absolute measurements with accuracy, since the recorded pressures vary with the radius of curvature of the globe, and with the resistance of the coats of the eye to the deforming force of the tonometer, but it is accurate for comparative measurements in the same individual at different times, or between the two (approximately equal) eyes of the same individual at the same time Since the pressures in the two eyes may thus be considered identical, the pressure was taken in the second eye by the saline-filled manometer, and the reading transposed to the first eye In the third experiment the tension was taken with a tonometer, and the venous pressure then observed, the manometer was then inserted, and after equilibrium had been established at the original intra-ocular pressure, the venous pressure was again taken, and a similar reading again obtained

The pressures recorded in three dogs were these (each the average of six readings)

No. of dog	I O P	V P	Difference V P — I O P
1	22 mm Hg (300 mm. citrate)	23.5 mm Hg (320 mm. citrate)	1.5 mm Hg
2	26 mm. Hg (350 mm. citrate)	28 mm Hg (380 mm. citrate)	2 mm Hg
3	25 mm. Hg (340 mm. citrate)	26 mm Hg (350 mm. citrate)	1 mm Hg

The venous pressure in the intra-scleral veins, therefore, is slightly above the intra-ocular pressure under normal conditions, the pressure difference averaging 1.5 mm Hg

The pressure in the extra-scleral veins The technique of Seidel⁽²⁰⁾ was adopted, a glass cylinder being used as a pressure-chamber whose base was formed of a membrane of softened cellophane, and which was in communication with a manometer and levelling bulb containing warmed saline. The intra-ocular pressure was taken as in the previous experiments both by tonometer and manometer. The pressure was estimated in the subconjunctival veins near the corneo-scleral junction that point being taken as standard of measurement when the first signs of interference with the blood flow became apparent. This precaution is necessary in order to eliminate the factor of hyperæmia, since, if the pressure be maintained, the vein originally emptied of blood refills and begins to pulsate, and a vessel which originally required 15 mm Hg to obliterate it, now requires a pressure of 20 mm Hg. Over a series of 12 experiments on dogs results comparable to those of Seidel were obtained, although they were slightly higher. The pressure in the veins soon after their exit from the eye varies from 5 to 8 mm Hg below the intra-ocular pressure, the average figure being 7.2. It may be repeated that the interpretation put upon these results by this observer is not admitted, viz. that they represent the intra-ocular venous pressure, the rapid drop in magnitude after leaving the eye is only to be expected, the pressure level falling to that obtaining generally in the veins of the orbit and head.

The pressure in the intra-ocular veins The retinal circulation is, except for very minor anastomoses, anatomically separated from the choroidal, but, since the venous pressures throughout the eye will be shown to vary with and be dependent upon the intra-ocular pressure, it would seem probable that the exit pressures throughout the eye are to all intents and purposes equal. Although, for anatomical reasons, the pressures in the capillaries of the two circulations are unequal, there is a large amount of evidence which goes to show that the entrance and exit pressures are similar, and that the same pressure gradient exists in both systems, although it is unequally proportioned. In the iris of many animals there are vessels of such a size as to be observed readily through a binocular loupe. On applying graduated amounts of pressure to the globe of the eye by means of a dynamometer, these eventually pulsate and finally are obliterated as the diastolic and then the systolic pressures are reached. These changes take place in the iris *pari passu* with similar

changes in the retina This was first noted by Leplat⁽¹³⁾ in the dog, and Bonnefon⁽⁵⁾ in the rabbit, while Magitot and Bailliart⁽²³⁾ noted the same relation in the cat Bleidung⁽⁴⁾, by compressing the eye by a pressure chamber through which ophthalmoscopic examination was possible, concluded that in man the circulatory conditions in both retinal and chorioidal systems varied together, and, taking advantage of a case of a vascularised persistent pupillary membrane, Vossius⁽³²⁾ noted the parallelism in the behaviour of the vessels therein with those of the retina

The intra-ocular venous pressure was therefore studied in the retinal vein, inasmuch as it is easily observable by means of the ophthalmoscope, and is less readily influenced by a delicate vaso-motor mechanism which reacts to any intra-ocular manipulation by an immediate response than are the veins of the iris and ciliary body The relation between the intra-ocular pressure and the venous pressure was determined by the establishment of a fistula between a vein on the optic disc and the cavity of the eye The outer canthus was slit up, and a very fine needle with a knife point inserted through the sclera behind the ciliary body and lens into the vitreous Guided by observation through the ophthalmoscope, the point of the needle was approximated to the termination of a retinal vein upon the optic disc, and then the handle was carefully supported for 15 minutes until any pressure reaction due to the introduction of the instrument had subsided That any such reaction is small, provided the needle is sharp enough and small enough, was demonstrated by repeated controls carried out with a manometer inserted into the eye Still under direct vision through the ophthalmoscope, the slightest movement given to the needle now suffices to pierce the wall of the vein with the knife point It is easy to differentiate an artery from a vein, and even if a mistake is made, the result is at once apparent on piercing an artery the fundus at once fills with blood, in the case of a vein a small jet of blood flows out more slowly, and forms, initially, a cone in the vitreous in the track of the needle This would seem to happen invariably in dogs, in cats, and in rabbits since it was tried in a large number of animals, 25 in all, with an identical result Since the blood flows out of the vein, the venous pressure is higher than the intra-ocular, and any deviation from normal caused by the slight movement of the needle will act in the direction of raising the ocular pressure, and so confirm rather than vitiate the result

That the difference between the two is not large is seen in the occurrence of a spontaneous venous pulse at the disc in many individuals and

animals Normally there is a pressure pulse in the eye of an amplitude of a little over 2 mm Hg, corresponding with the systole of the retinal artery, and showing a sudden ascent, a sustained plateau, and a slow descent Alternating with this the veins at the disc may show an inverse pulsation, being compressed at the summit of the arterial systole, and in many cases, where this is absent normally, the application of a small amount of pressure (2-3 mm. Hg) to the globe is sufficient to induce it In other cases increased pressure on the globe fails to induce a pulse at all, in these, however, the same pressure relation holds good, as is seen by measurement of the intra-scleral venous pressure, and by the establishment of a fistula in a retinal vein Bailliar's conclusions, which were reached on very insecure grounds, cannot therefore be accepted, for the occurrence of a venous pulse at the disc depends on factors more complicated than pressure differences alone, thus it tends to be abolished by the neutralising effect in the optic nerve of the pulsation of the retinal artery, alongside which vessel the vein runs in close association for some distance, or by any condition which tends to obstruct the unimpeded flow of blood, as, for example, venous engorgement (Helfreich(12)), or by marked rotation of the eyes to the side (Graves(10)), a movement which will kink the vein Moreover, the external pressure which must be applied to the eye to induce a non-spontaneous pulse is no accurate measure of the normal difference between the venous and the intra-ocular pressures, for, since the one varies with the other, the latter cannot be approximated to the former without altering it also, thus leaving the initial pressure difference between them quite unknown.

The variation of venous pressure with intra-ocular pressure It has been established in a general way that the intra-ocular pressure varies very closely with the venous pressure On tying the vortex veins as they issue from the eye very large intra-ocular pressures, up to 80 and 90 mm Hg, are registered Ligature of a single vein produces the same effect to a lesser degree, as we have seen in discussing the experimental technique of Weiss and Lullies, ligature of all the veins produces in a short time a shallow anterior chamber, a dilated pupil, a hyperæmic iris, and engorged and swollen vessels throughout the eye—later the cornea becomes opaque, the tension remains stony hard, and further ophthalmoscopic examination becomes impossible These effects find ample confirmation in the observations of several workers, the majority of whom were investigating the effect of the venous circulation on glaucoma—Adamùh(1), Ulrich(31), Leber(17), Bartels(3), Weber(33), & Schul-

tén(20), Koster(10) and Magitot(21) The somewhat equivocal results that have been obtained on ligation of the jugular veins are readily explained when the extremely free anastomoses are taken into consideration, thus Adamuk(1), Graser(9), v Schultén(20) and Parsons(25) produced only a slight increase of intra-ocular pressure thereby When, however, the channels are all simultaneously impeded the result is more marked, as on passing a ligature round the neck (Bonnefon(6)), or by compressing the thorax (Mazzei(24)), while L Hill(13), by compressing the vena cava, demonstrated that a rise of pressure here was followed by a proportionate rise in the intra-ocular pressure A large amount of clinical evidence, moreover, is available on the production of glaucoma by the obstruction of the efferent veins by traumatic, thrombotic, or inflammatory processes Seefelder(27), Magitot(20), Christel(6), Heerfort(11), Stähli(30), Ischreyt(15), and others

A similar relation obtains on raising the intra-ocular pressure A mercury manometer with a levelling bulb was connected with the eye of a cat, and the behaviour of the veins of the retina watched as the pressure was raised As the intra-ocular pressure rises the veins show no compression until a pressure of 65-70 mm Hg is reached, when they begin to be slightly reduced in size, at higher pressures—70-80—when the flow through the arteries is beginning to become intermittent owing to the diastolic pressure being overcome, they become definitely constricted, but they are not obliterated until a pressure of 115 mm Hg is reached—a pressure which stopped the flow in the arteries, and very nearly approximated the carotid level of 120 mm Hg At this point ophthalmoscopic examination reveals that the veins, which in the lower stages of pressure increment had been engorged, have now been reduced to mere streaks, the contained blood taking up a granular appearance as circulation ceases

In order to determine the relative values during the variation of pressure, the venous pressure was measured in the intra-scleral veins by the capillary manometer already described, while the intra-ocular pressure was raised by a mercury manometer inserted into the eye. On raising the intra-ocular pressure, the pressure in the veins just before their exit from the sclera was observed to fall slightly below that obtaining in the eye thus with an intra-ocular pressure of 40 mm Hg the venous pressure at this point was found to be 39 mm Hg At the same time ophthalmoscopic examination showed the intra-ocular veins to be engorged A needle was then introduced into the posterior part of the eye as in the previous series of experiments, and the same result obtained

as formerly on the establishment of a fistula—a hæmorrhage into the eye—demonstrating that the intra-ocular venous pressure was still higher than the chamber pressure. A further reading of the intra-scleral venous pressure was then made, and the results confirmed the previous finding that the pressure at this point was slightly less than the artificial pressure maintained in the eye.

This would seem to have an important bearing on the physiology of the intra-ocular pressure and the mechanism called into action to maintain it at a normal level. The results obtained suggest that the venous pressure in the eye is always slightly higher than the chamber pressure, and that, while there is a pressure decrement in the veins through the scleral coat, normally the pressure at the exit is still in excess of that in the eye. This organ thus falls into line with the rest of the body wherein the venous pressure is slightly higher than the tissue pressure. Owing to the delicate nature of the venous walls which could withstand no degree of pressure from outside without collapsing, it is an essential postulate for the maintenance of a continued circulation that the arterial pressure should be higher than the capillary, the capillary higher than the venous, and the venous higher than the chamber pressure. When the intra-ocular pressure is raised the circulatory system is compressed. That part with the lowest lateral pressure will give way first, that is, the veins at their point of exit will tend to be obliterated. As soon as this occurs the blood flow will be checked, the *vis a tergo* from the arteries will pile up pressure, the constriction will be forced open, and the circulation will proceed at a higher pressure level. This process will repeat itself in a cumulative manner until the available force from the arteries is exhausted, that is, until the pressure of the ophthalmic artery has been reached, at which point the entire circulation will cease, and the vessels will be obliterated. In the meantime, the pressure at the venous exits inside the eye has approximated to the chamber pressure, the pressure decrement in the vessels through the sclera, with their lumen kept always patent by the investing scleral tissue, still obtains, and the pressure in the intra-scleral veins near the outside of the ocular coat now falls below the intra-ocular pressure. The Canal of Schlemm, situated favourably as it is in the middle of the thickness of the sclera, i.e. in the middle of this pressure decrement, will now acquire a pressure less than the chamber pressure, a hydrostatic flow will be set up, draining off aqueous, lowering the intra-ocular pressure, and acting as a safety-valve tending to restore the normal pressure conditions of the eye.

CONCLUSIONS

1 Methods are described for the measurement of the venous pressure of the eye for which is claimed a closer approximation to the normal conditions than is obtained by methods employed hitherto

2 Normally the venous pressure in the eye and in the vessels passing through the coats of the eye is slightly higher (1-2 mm Hg) than the intra-ocular pressure, immediately on leaving the eye there is a rapid fall

3 The venous pressure varies directly and very intimately with the intra-ocular pressure

4 On a rise of intra-ocular pressure, the venous pressure within the sclera, and therefore the pressure in the Canal of Schlemm, falls below the intra-ocular pressure

5 Under normal conditions the aqueous is absorbed into the capillary-venous stream by osmotic forces alone, under conditions of raised pressure a hydrostatic outflow may occur

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THE SECRETION OF PANCREATIC JUICE

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SINCE 1902 the secretin hypothesis of Bayliss and Starling⁽¹⁾ for the secretion of pancreatic juice has been generally accepted. This hypothesis states that secretin is derived from a precursor (prosecretin) by the action of acid, and prosecretin exists in that situation in which it is in a position to be acted upon by acid chyme and to discharge into the blood the substance which acts as a timely stimulus to the pancreatic cells. As a corollary to this hypothesis the secretion of pancreatic juice has been causally associated with the secretion of hydrochloric acid by the gastric mucosa. In a previous paper by Mellanby and Huggett⁽²⁾ it has been shown that secretin exists in a preformed condition in the mucous membrane of the small intestine since active solutions of it may be obtained by solvents so diverse as water, 5 p.c. NaCl, 85 p.c. NaCl, 2 p.c. HCl, 1 p.c. NaOH, 75 p.c. alcohol and 75 p.c. acetone. Further, it has been shown that secretin exists in the preformed condition in the mucous membrane of the small intestine in positions far removed from any possible action of acid chyme. It is evident, therefore, that there exists no immediate causal relation between gastric acidity and pancreatic secretion.

According to Bayliss and Starling the formation of secretin from prosecretin by the action of acid chyme leads, *ipso facto*, to its discharge into the blood and thereby acts as a timely stimulus to the pancreatic cells. But since secretin exists in a preformed condition in the intestinal mucosa it is evident, assuming that secretin is immediately responsible for the secretion of pancreatic juice, that some definite event must cause the passage of this secretin from the cells of the intestinal mucosa into the portal blood so as to evoke the secretion. A detailed investigation was made in order to determine whether the entry of acid from the stomach into the duodenum acts as the timely stimulus or whether some other alimentary stimulus causes the absorption of secretin into the blood.

Experimental methods The experiments were carried out on cats anaesthetised by urethane (1.5 gm. per kilo. of body weight). The

pancreatic juice was collected from a cannula tied into the pancreatic duct, and the rate of secretion was determined by timing the drops. Intravenous injections were made by means of a cannula tied into the right femoral vein.

Previous work The secretion of pancreatic juice may be evoked by the introduction of numerous substances (apart from the normal food stuffs) into the stomach or duodenum. Thus Dolinski⁽³⁾ observed in a dog that a copious secretion was produced on passing 200 c.c. of 5 p.c. HCl into the stomach and that the greater the concentration of acid the greater the secretion of pancreatic juice. Further, he observed that the action of hydrochloric acid was not specific since other acids, such as lactic, phosphoric and acetic acids could produce this effect. Even water in copious quantities was effective and Bekker⁽⁴⁾ showed that mineral waters containing a large quantity of CO₂ were especially so. The introduction of acid into the stomach is generally recognised as the strongest alimentary stimulus for pancreatic activity, but many other substances have been observed to produce this action, notably fat and alcohol (Dolinski), soaps (Babkin⁽⁵⁾), soap into the duodenum (Fleig⁽⁶⁾), chloral hydrate, mustard oil, croton oil (Wertheimer and Lepage⁽⁷⁾). It is noteworthy that, as Bayliss and Starling showed, secretin introduced directly into the duodenum does not cause a secretion of pancreatic juice.

At the beginning of this work the capacities of a large number of substances, including many of those mentioned above, to cause a secretion of pancreatic juice, when introduced directly into the duodenum, were determined. Quantities up to 50 c.c. of each of the following substances were injected into the duodenum immediately below the entrance of the pancreatic duct: secretin at different reactions (*pH* 6.5, 7.0 and 10.5), secretin in 75 p.c. alcohol, 1 p.c. sodium bicarbonate, fresh pancreatic juice, 10 p.c. glycerol, water, 1 p.c. suspension of mustard, 1 p.c. croton oil in olive oil, and 1 p.c. HCl. The only solution which caused a secretion of pancreatic juice was 1 p.c. HCl. The quantity secreted after the introduction of 50 c.c. of 1 p.c. HCl into the duodenum was ten drops in 10 minutes. Since the intravenous injection of 1 c.c. of an active solution of secretin may stimulate the pancreas to secrete 50 drops of juice in the course of half an hour it is evident that even 1 p.c. HCl cannot be looked upon as an adequate alimentary stimulus for the secretion of pancreatic juice. A series of experiments was also carried out in which extracts of the various parts of the alimentary canal, either alone or in conjunction with one another, were introduced into the

duodenum As a matter of interest the first experiment which gave a clue to the nature of the alimentary stimulus responsible for the secretion of pancreatic juice is detailed below

10 c c of a mixture containing 2 c c of ox bile¹ and 8 c c of a 2 p c HCl extract of the gastric mucosa of a cat was injected into the duodenum of an anaesthetised cat After a latent period of 7 minutes pancreatic juice started to drop from the cannula tied into the pancreatic duct The rate of secretion is shown in the following figures

Mins	Pancreatic juice in drops
7	1
13	10
17	20
25	40
52	80
75	100
120	120 (6 c c.)

The secretion of pancreatic juice continued for 3 hours At the end of that time 65 c c of juice had been collected It may be observed that within the first hour 4 c c of pancreatic juice was secreted, in the second hour 2 c c of juice and in the third hour 05 c c of juice As a control experiment 5 c c of the above mixture of bile and extract of gastric mucosa was injected directly into the blood stream. No secretion of pancreatic juice was produced, showing that the mixture did not directly stimulate secretion after absorption into the blood The amount of pancreatic juice secreted, and the rate of secretion indicated that the essential factors which constitute the alimentary stimulus for this secretion were present in this experiment

A number of experiments were carried out to determine whether in the above mixture it was the acid or some other constituent derived from the bile or gastric mucosa that was active As a result of these experiments it became evident that the essential factor was the introduction of bile into the duodenum, but that the efficiency of bile was intimately related to its reaction The extract of the gastric mucosa was not essential except in so far as in the above experiment it produced that reaction which was essential to the orderly action of bile

(A) *Bile as the alimentary stimulus for pancreatic secretion*

The introduction of bile of an adequate reaction into the duodenum produces a copious secretion of pancreatic juice This statement is exemplified in the following experiment

¹ Ox bile was used in all the experiments done with bile since a large quantity can be readily obtained and a series of comparable experiments may be made on one specimen.

A dilute solution of bile (2 c c bile, 8 c c 85 p c NaCl, 05 c c HCl (N)) was injected into the duodenum of a cat. The reaction of the mixture was approximately neutral (pH 6.9). After a latent period of about 5 minutes the secretion of pancreatic juice started and continued for about 2 hours. At the end of that time 5 c c of juice had been collected.

m.	s	Pancreatic juice in drops
5	40	1
7	10	5
9	30	10
13	35	20
24	22	40
36	34	60
65	13	80
95	26	100

The figures illustrate a striking fact which may be observed in a successful experiment, viz the rapid rate at which pancreatic juice is produced after the initiation of secretion. It may be seen in the above figures that after the initial latent period, juice was secreted at the rate of two drops a minute for the next 30 minutes. Even powerful extracts of secretin introduced directly into the blood rarely provoke in the cat a secretion at a greater rate than three drops a minute and at the end of about 20 minutes the secretion ceases. A series of experiments was now undertaken to determine whether bile contained an unknown substance which produced this secretion or whether the effect was due to one of its well-known constituents.

(a) *Bile salts* An experiment similar to that described was carried out except that a mixture of bile salts (sodium taurocholate and sodium glycocholate) was substituted for the bile. The following mixture was injected into the duodenum of a cat: 5 c c bile salts (5 p c), 5 c c 85 p c NaCl and 05 c c HCl (N). After a latent period of only 2 minutes the secretion of pancreatic juice started.

m	s	Pancreatic juice in drops
1	55	1
3	2	5
4	40	10
7	44	20
16	32	40
40	41	60 (29 c c)

It is clear, therefore, that bile salts, contained in a solution of adequate reaction, injected into the duodenum stimulate the secretion of pancreatic juice. In this case the secretion was preceded by a short

latent period (2 minutes) and was completed in a comparatively short period of time (45 minutes)

(b) *Cholic acid* The phenomenon was further analysed by determining the capacity of cholic acid to initiate the secretion of pancreatic juice

1 p c cholic acid (pure) was dissolved in 85 p c NaCl by means of a trace of NaOH (N) After solution the fluid was brought to the acid side of neutrality by the addition of HCl (N) The amount of acid added did not precipitate the cholic acid but made the solution slightly opalescent 10 c c of this 1 p c cholic acid solution was injected into the duodenum of a cat, with the following result

m.	s.	Pancreatic juice in drops
4	0	1
6	50	5
9	35	10
16	10	20
28	15	30
43	40	35 (17 c.c.)

It is evident that pure cholic acid is able to act as an alimentary stimulus for the secretion of pancreatic juice, although its capacity to act thus is less than that of bile salts and these in turn less than that of pure bile

(c) *Bile pigments* The injection of 4 c c of an alcoholic extract of bile pigment, in a concentration equal to that contained in the original bile, into the duodenum of a cat did not produce a secretion of pancreatic juice

(d) *The function of mucin in bile* From the above results it is evident that cholic acid is the essential constituent of bile which acts as the alimentary stimulus for the secretion of pancreatic juice but that the action of cholic acid is profoundly modified by the state in which it exists in the bile The combination of cholic acid with glycine and taurine is one of the modifying factors but another factor, at least as important, is the presence of the mucin in the bile The function of mucin is illustrated by the following experimental results obtained from a cat into the duodenum of which was first injected (1) dilute bile (2 c c bile, 8 c c 85 p c NaCl, 05 c c HCl (N)), and later (2) the same mixture but made with bile from which the mucin had been precipitated The following figures give the rates and the quantities of juice secreted under the two conditions

Pancreatic juice in drops	(1)		(11)	
	h	m	h	m
1		54		22
5		58		29
10	1	16		34
20	1	11		47
40	1	32		57
90	2	20	1	5
120	2	53	—	—

(1) The secretion continued for 4 hours. At the end of that time 12 c.c. of pancreatic juice had been produced. (11) The secretion continued for 2 hours only. At the end of that time 54 c.c. of pancreatic juice had been produced. This experiment illustrates the long latent period which may elapse between the introduction of the bile into the duodenum and the initiation of pancreatic secretion. It further emphasises the importance of mucin in the bile in prolonging pancreatic secretion. The results indicate that mucin prevents a too rapid absorption of the bile salts and thereby prolongs the action of the bile as an alimentary stimulus for the secretion of pancreatic juice.

As a subsidiary action of mucin it may be observed that the injection of bile salts into the duodenum occasionally causes hæmoglobinuria, a result which is probably due to the too rapid absorption of the bile salts causing the hæmolysis of red corpuscles. Probably the association of mucin with bile salts in bile prevents this toxic action. In fact mucin carries to a still further stage the effect produced by the combination of cholic acid (the essential stimulus) with glycine and taurine.

(e) *The influence of the ligature of the bile duct and pylorus on the secretion of pancreatic juice.* The secretion of pancreatic juice after the injection of bile into the duodenum continues for a considerable period of time, in the above experiment, for instance, for 1 hour. Experimental results of this nature indicated that the continuance of secretion might be due to the occurrence of secondary events, such as the renewed entrance of bile into the duodenum from the gall bladder or a repeated acid stimulus from the stomach in consequence of the opening of the pyloric sphincter. Experiments were therefore made to test these possibilities.

After the ligature of the common bile duct, 10 c.c. of dilute slightly acidified bile (2 c.c. bile, 8 c.c. 85 p.c. NaCl, 05 c.c. HCl(N)) was injected into the duodenum of a cat. A copious secretion of pancreatic juice (1), was produced during the succeeding 3 hours, and then ceased. After the lapse of an hour the pyloric sphincter was securely tied and an

additional 10 c c of dilute bile, (u), similar to the above was injected into the duodenum. The results are given below

Pancreatic juice in drops	(i)		(u)	
	m.	s.	m.	s.
1	18	4	9	45
10	25	31	21	10
20	29	54	31	55
40	38	3	46	49
80	51	5	71	35
120	65	0	130	9
Total juice secreted	11 c c.		53 c c	

Certainly the pancreatic juice was secreted more slowly after tying the pylorus but too much importance must not be attached to this fact since the experiment had lasted 5 hours before secretion (u) was initiated. The results offer fair evidence that the long continued secretion of pancreatic juice after the introduction of bile into the duodenum is not causally associated with the secondary passage of bile from the cat's own gall bladder into the duodenum or with the entrance of acid chyme from the cat's stomach. At the conclusion of the above experiment 1 c c of a solution of secretin was injected into the femoral vein. A copious secretion of pancreatic juice lasting for 20 minutes was elicited, showing that the action of secretin when introduced directly into the blood stream is not due to the entrance of bile into the duodenum from the cat's gall bladder—a possible action which required to be controlled by direct experiment.

(f) *The minimal quantity of bile* Experiments were carried out to determine whether there was any relation between the quantity of bile injected into the intestine and the amount of pancreatic juice secreted. It was found that cats vary greatly in the amount of secretion produced after the injection of dilute bile into the duodenum. The following experiment indicates that the amount of pancreatic juice secreted in any cat is determined to some degree by the quantity and dilution of the bile which enters the intestine.

10 c c of the dilute bile (0.5 c c bile, 9.5 c c 85 p c NaCl, 0.2 c c HCl (N)) was injected into the duodenum of a cat. After the cessation of the flow of pancreatic juice 10 c c of a more concentrated solution of bile (5 c c bile, 5 c c 85 p c NaCl, 0.5 c c HCl (N)) was injected into the duodenum. The rates of secretion in the two cases are given below.

The figures show that the amount of bile injected into the duodenum determines (a) the latent period, (b) the duration of secretion and (c) the amount of pancreatic juice secreted. A similar experiment on another

Pancreatic juice in drops	Dilute bile minutes	Stronger solution of bile minutes
1	20	7
10	28	13
20	32	18
40	41	29
60	53	39
80	53	50
120	—	70
160	—	97
200	—	150
Total juice secreted	43 c c	12 c c

cat showed that 2 c c of bile diluted to 10 c c produced a secretion almost identical with that caused by 5 c c bile diluted to 10 c c. It is of interest to observe that the average capacity of a cat's gall bladder is approximately 2 c c, so that dilution of this bile with the gastric contents would produce conditions of the same order as that observed in the above experiments.

(B) *The influence of the reaction of bile*

Small changes in reaction exert a profound influence on the capacity of bile to act as the alimentary stimulus for the secretion of pancreatic juice. On the other hand, the mechanism appears to be completely free from the control of the autonomic nervous system. The importance of the reaction of bile on its capacity to act as the alimentary stimulus for the secretion of pancreatic juice was demonstrated at an early stage in the investigation. In the experiments previously described the reaction of the dilute bile injected into the duodenum was approximately neutral or just on the acidic side of neutrality. In a number of the earlier experiments, however, it was found that the injection of bile of this reaction led to no secretion of pancreatic juice. In some few cases the absence of secretion was due to the occlusion of the pancreatic duct, and in other cases the duodenum was full of gross parasites. But, excluding all these cases, it was evident that some unknown factor entered into the mechanism. A series of experiments showed that the state of digestive activity of the alimentary canal was the deciding factor. If active gastric digestion was proceeding in the cat the effective alimentary stimulus for calling out a secretion of pancreatic juice was the presence of dilute acidic bile in the duodenum, if, however, the cat was in a fasting condition, the injection of a slightly alkaline bile into the duodenum was essential. A cat fed with minced beef the previous afternoon and given milk at 7 a m, regularly gave a large and prolonged secretion of pan-

creatic juice when dilute acidic bile was injected into its duodenum after midday (urethane having been administered at about 9 a m) This is illustrated by the experimental results recorded on p 422 On the other hand, a cat supplied with water only for the previous 24 hours produced no secretion of pancreatic juice when acidic or neutral bile was injected into the duodenum, but gave a copious secretion if a definitely alkaline solution of bile was first injected. The following experimental results illustrate this latter statement

Cat supplied with water only for previous 24 hours The following mixture was injected into the duodenum 2 c c bile, 7 c c 85 p c NaCl, 1 c c NaHCO_3 (15 p c) The amount of bicarbonate added to the dilute bile corresponds to the quantity which may be assumed to be present in the arterial blood of a cat, calculated from the CO_2 content After a latent period of 26 minutes pancreatic juice was secreted at a fairly rapid rate for the next 2 hours

Pancreatic juice in drops	Minutes
1	26
20	40
40	52
60	64
80	76
100	90
120	110
140	130
<hr/>	
Total juice 67 c.c	

The mechanism has been analysed by means of a considerable number of experiments Briefly stated it has been found that in a fasting cat the optimum reaction for the dilute bile injected into the duodenum is approximately pH 7.8, and in a cat undergoing active gastric digestion, the optimum reaction is approximately pH 6.5 The results emphasise in a marked way the dependence of alimentary processes on reaction

A further series of experiments was carried out to determine how far the reaction of the bile injected into the duodenum influenced its capacity to produce a secretion of pancreatic juice after secretion had been initiated It was found that in a fed cat, after secretion had been started by an injection of bile of adequate reaction into the duodenum, subsequent injections of bile of considerable degrees of acidity were able to evoke a further secretion of pancreatic juice This fact is illustrated by the following experiment

10 c c of dilute bile (5 c c bile, 5 c c NaCl 85 p c, 0.5 c c HCl(N)), of a reaction pH 7, was injected into the duodenum of a fed cat At the

conclusion of the secretion evoked by this bile a similar dilution of bile, to which HCl was added until the reaction was pH 4.5, was now injected into the duodenum. The respective rates of secretion were as follows

Pancreatic juice in drops	Dilute bile (pH 7)		Dilute bile (pH 4.5)	
	(i)		(ii)	
	h.	m.	h.	m.
1	7		2	
10	13		6	
20	18		11	
40	29		19	
60	39		27	
100	1	3	1	20
200	2	28	—	—
Total juice	12 cc		7 c.c.	

It may be observed that (a) the second injection of acidic bile stimulated the secretion of a considerable quantity of pancreatic juice, although the total quantity was less than that secreted under the stimulus of the neutral bile, and (b) the initial rate of secretion with the acidic bile was greater than that produced after the injection of the neutral bile.

Similar results may be observed with the fasting animal. Although in the cat with the resting alimentary canal, the bile injected into the duodenum which initiates the secretion of pancreatic juice must be on the alkaline side of neutrality, subsequent injections of bile may be definitely acidic and yet cause a renewed secretion of pancreatic juice. This statement is illustrated in the following experimental results. Column 1 shows the rate of secretion of pancreatic juice secreted after the injection of dilute alkaline bile into the duodenum (2 c.c. ox bile, 7 c.c. NaCl, 85 p.c. and 1 c.c. NaHCO_3 15 p.c.). Column 2 shows the rate of secretion produced in the same cat by the injection of dilute acidic bile into the duodenum after secretion 1 had ceased (2 c.c. ox bile, 8 c.c. NaCl 85 p.c., 1 c.c. HCl (N)).

Pancreatic juice in drops	(i) minutes	(ii) minutes
1	6	5
10	11	10
20	14	13
40	20	20
80	32	43
120	54	74
160	95	—

The similarity between the rates of secretion in the two cases is remarkable and indicates that the activity of the intestinal epithelium in passing secretin into the portal blood is determined by its own

mechanism rather than by the relation of the reaction of the intestinal contents to the reaction of the blood. These and many similar experiments indicate that although the bile injected into the alimentary canal must be of an adequate reaction, depending on the state of digestive activity of the animal, to initiate the secretion of pancreatic juice, yet the secretory mechanism having been set in motion, subsequent injections of bile may vary widely in reaction and yet continue to act as adequate stimuli for the secretion of pancreatic juice.

(C) *The mechanism of the alimentary action of bile*

The mechanism whereby the introduction of bile into the duodenum leads to a secretion of pancreatic juice was investigated. The experimental analysis indicated that the absorption of bile salts from the intestine determines the passage of secretin from the cells of the mucous membrane into the blood and thereby to the pancreas.

(a) *The effects of introducing cholic acid into the duodenum and ileum respectively*. Cholic acid was dissolved in dilute NaOH and then made slightly acidic by the addition of HCl. The acidity was such as to render the solution slightly opalescent but not to cause precipitation. Columns (i) and (ii) show respectively the rates of secretion after the injection of 10 c.c. of 1 p.c. cholic acid solution into the duodenum and ileum.

Pancreatic juice in drops	(i) m. s.	(ii) m. s.
1	4 0	15 0
5	6 50	—
10	9 35	—
20	16 10	—
30	28 15	—
40	58 0	—

Thus the injection of 10 c.c. of cholic acid (1 p.c.) into the duodenum produced 2 c.c. of pancreatic juice in 1 hour, a similar quantity injected into the ileum of the same animal produced one drop of pancreatic juice. The cholic acid appeared to be absorbed in both cases. Since secretin is present in maximal concentration in the mucous membrane of the duodenum and practically absent from the ileum, the result offers strong evidence in favour of the hypothesis that the absorption of bile acids from the intestine causes the passage of secretin into the portal blood and hence leads to the secretion of pancreatic juice. Confirmatory evidence in favour of this hypothesis was obtained from experiments in which the effects were determined of vagal and sympathetic paralysis.

on the secretion of pancreatic juice produced by (1) the injection of secretin into the blood, and (2) the injection of bile into the duodenum

(b) *Paralysis of the vagus* In a previous paper(s) the effect of paralysis of the vagus on the secretion of pancreatic juice produced by the injection of secretin directly into the blood has been dealt with in detail. Briefly stated, paralysis of the vagus by atropine diminishes the enzyme content but increases the rate of secretion of pancreatic juice produced by the injection of secretin into the blood. The following experimental results show the effects produced by atropine injected into the blood stream during the secretion of pancreatic juice subsequent to the injection of bile into the duodenum.

10 c c of dilute bile (2 c c bile, 8 c c 85 p c NaCl, 05 c c HCl (N)) was injected into the duodenum. After a latent period of 5 minutes pancreatic juice was rapidly secreted. After the secretion of 4 c c of juice, 5 mg of atropine was injected into the femoral vein. As a result of this procedure the rate of secretion was accelerated by one third

	Pancreatic juice in drops	m.	s.
(i) Before injection of atropine	{ 80	65	13
	{ 84	71	4
	{ 88	77	15
(ii) After injection	{ 98	93	21
	{ 102	97	30
	{ 106	102	10
	{ 110	105	20
	{ 144	109	17

The acceleration of the rate of secretion of pancreatic juice produced by atropine persists for a considerable period of time—in this experiment for more than 30 minutes. The duration of the acceleration indicates that atropine not only dilates the pancreatic ducts by paralysis of the vagal endings in the smooth muscle surrounding them (Anrep⁽³⁾) but also affects the glandular tissue of the pancreas. As a confirmatory experiment the relative rates of secretion of pancreatic juice produced by two separate injections of dilute bile into the duodenum, (i) before, and (ii) after the intravenous injection of 10 mg of atropine were determined.

Pancreatic juice in drops	(i) m s	(ii) m s
1	5 40	20
10	9 30	6 25
20	13 35	11 54
40	24 22	20 0
60	36 40	28 12

The acceleration of pancreatic secretion produced after the intravenous injection of atropine by bile in the duodenum is particularly marked in this experiment. A control experiment in which atropine was injected directly into the intestine showed that the effect was not due to a local action of atropine on the intestinal mucosa facilitating the absorption of the injected bile into the blood stream from the duodenum.

(c) *Paralysis of the sympathetic* An experiment similar to the above was carried out before and after the paralysis of the secretory fibres of the sympathetic by the intravenous injection of 1 mg of ergotamine tartrate.

10 c.c. of dilute bile (2 c.c. bile, 8 c.c. NaCl 85 p.c., 05 c.c. HCl (N)) was injected into the duodenum. Then 20 minutes after the initiation of pancreatic secretion 1 mg of ergotamine tartrate was injected into the femoral vein.

	Pancreatic juice in drops	m	s
(i) Before injection of ergotamine	28	11	36
	32	15	15
	36	17	44
	40	20	30
(ii) After injection	44	23	30
	48	26	55
	52	31	8
	56	34	52
	60	37	47

It is evident that paralysis of the sympathetic secretory fibres by ergotamine tartrate has no effect on the secretion of pancreatic juice produced after the injection of bile into the duodenum. The result is similar to that observed after the intravenous injection of ergotamine tartrate on the secretion of pancreatic juice produced by the intravenous injection of secretin. It is a matter of considerable interest that the capacity of bile to act as an alimentary stimulus for the secretion of pancreatic juice is not diminished by paralysis of the vagus or the motor fibres of the sympathetic. The facts indicate that the absorption of bile salts from the intestine and the passage of secretin from the cells of the mucosa into the portal blood are independent of extraneous nervous influences to at least as great an extent as the action of secretin, injected directly into the blood, on the cells of the pancreas.

(D) *The cessation of secretion*

In the previous pages the factors determining the capacity of bile to act as an adequate alimentary stimulus for the secretion of pancreatic

juice have been discussed. The processes which determine the duration of pancreatic secretion after the injection of a given quantity of bile into the duodenum have been subjected to experimental analysis. Four possible causes have been investigated, (a) the exhaustion of secretin from the intestinal mucosa, (b) the presence of pancreatic juice in the intestine, (c) the rate of absorption of bile salts, and (d) the limited distribution of secretin.

(a) The amount of secretin contained in the intestinal mucosa is so large that there appears to be no diminution in its content after the injection of dilute bile into the intestine and the consequent production of a large quantity of pancreatic juice. Thus the mucous membrane of a cat which had secreted 21 c c of pancreatic juice after the injection of dilute bile into the duodenum was extracted with absolute alcohol in the usual way. This secretin extract (1 c c) injected into the femoral vein of an experimental cat stimulated the pancreas to secrete 4 c c of juice in 25 minutes. In fact a copious pancreatic secretion produced by the injection of bile into the duodenum appeared to be associated with the presence of an unusually large quantity of secretin in the duodenal mucous membrane.

(b) The influence of reaction on the capacity of bile to act as an alimentary stimulus for pancreatic secretion indicated that the cumulative action of the alkali contained in the pancreatic juice secreted into the duodenum would automatically stop the stimulating action of the bile. In order to examine this hypothesis, dilute bile was injected into the duodenum of a cat. A copious secretion of pancreatic juice was produced. At the end of 1 hour the total juice secreted from the pancreatic cannula was reinjected into the duodenum and the effect of this procedure on the flow of pancreatic juice was observed. The following figures show the rate of secretion (i) before, and (ii) after the injection into the duodenum of the secreted pancreatic juice.

Pancreatic juice in drops		Minutes
(i)	{ 80	43
	{ 100	52
(ii)	{ 120	61
	{ 140	80
	{ 160	110

The pancreas secreted at a slightly slower rate after the injection of the pancreatic juice into the duodenum, but as the secretion continued for a further period of an hour (at the end of which time the total juice secreted during the experiment amounted to 8.6 c c) it is evident that

the alimentary mechanism is not stopped by the entrance of the secreted pancreatic juice into the duodenum

(c) The absorption of the bile is not completed in the upper small intestine. After the cessation of pancreatic secretion, produced by the injection of bile into the duodenum, demonstrable quantities of bile salts may be found not only in the ileum but also in the large intestine. Therefore the early absorption of bile salts cannot be responsible for the cessation of pancreatic secretion.

(d) It is probable that the action of bile as the alimentary stimulus for the secretion of pancreatic juice is limited by the distribution of secretin in the intestinal mucosa. Thus in the case of the herbivorous animals, *e.g.* the goat, the distribution of secretin extends to the large intestine, as the following figures show

Small intestine (upper third)	100 units of secretin
„ (middle third)	90 „
„ (lower third)	18 „
Large intestine	8 „

In the case of the pig the distribution of secretin is limited to the first 10 feet of the small intestine, thus

Distance from pyloric sphincter in feet	Units of secretin
1	100
2	76
3	56
4	48
5	24
6	16
8	7
10	4

In the cat practically the whole of the secretin is contained in a few inches of duodenum, the jejunum and ileum containing only small quantities

Duodenum	100 units of secretin
Jejunum	17 „
Ileum	6 „

It is evident therefore that wide differences in the distribution of secretin occur in different animals. The extension of secretin into the mucous membrane of the large intestine in the goat would appear to be correlated with the continuous digestion which occurs in herbivorous animals.

The experimental analysis of the question indicates that the duration

of secretion of pancreatic juice after the entry of bile into the duodenum is determined by the distribution of secretin in the intestine rather than by changes of reaction, absence of bile salts or exhaustion of secretin from the cells of the intestinal mucosa. On this hypothesis it is evident that a large secretion of pancreatic juice might be evoked by a diminished peristalsis of the alimentary canal or the entrance of a large quantity of bile into the duodenum. In this connection it is clear that the volume of fluid in which the bile is contained is of some importance. Thus a well-marked secretion of pancreatic juice may be produced after the injection into the duodenum of 5 c c of bile diluted to 10 c c, whereas the same quantity of bile diluted to 4 c c produces a small secretion only. This effect of volume on the efficacy of a given quantity of bile injected into the duodenum on the rate of pancreatic secretion has not, however, been worked out in detail.

SUMMARY

(1) The introduction of bile of an adequate reaction into the duodenum of a cat causes a copious secretion of pancreatic juice.

(2) The active substance present in bile is cholic acid, but the activity of cholic acid is profoundly modified by (a) its association with taurine and glycine, and (b) the presence of mucin in the bile.

(3) The optimum reaction of bile as a pancreatic stimulant varies with the state of digestion of the animal. In a fasting cat the optimum reaction is pH 7.8, in a cat in which gastric digestion is actively proceeding the optimum reaction is pH 6.5.

(4) Paralysis of the vagus by the intravenous injection of atropine or paralysis of the motor side of the sympathetic system by the intravenous injection of ergotamine does not diminish the capacity of bile to act as an alimentary stimulus for the secretion of pancreatic juice.

(5) The duration of pancreatic secretion after the injection of a given quantity of bile into the duodenum is determined mainly by the distribution of secretin in the intestinal mucosa.

(6) The immediate stimulus for the secretion of pancreatic juice is secretin. Secretin contained in the cells of the intestinal mucosa is carried into the portal blood associated with the bile salts contained in the fluid absorbed from the intestine.

(7) The facts offer an adequate basis for the appreciation of the severe digestive disturbances which occur in catarrhal jaundice.

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THE SPLEEN AND THE RESISTANCE OF RED CELLS By D ORAHOVATS¹

(From the Physiological Laboratory, Cambridge)

BARCROFT and others(1) showed that the spleen may be regarded as a reservoir for blood which is emptied into the general circulation in cases when the body requires more hæmoglobin, this reservoir is filled again with blood when such a need ceases to exist. The only source from which the spleen may refill itself is the blood of the general circulation which is supplied to it. A part only of the passing blood is detained and this part may have been selected by the spleen out of the blood of the general circulation, or it may have been detained without any selective choice. If it is selected it must differ in some way from the blood of the body. The spleen has some influence upon the red cells, R M Pearce with Krumbhaar and Frazier(2) have shown for instance that the resistance of the red cells of the blood from the general circulation to hypotonic salt solutions is greatly increased after splenectomy.

The experiments described below were performed to investigate the resistance of the red cells of the spleen pulp itself compared to the resistance of the red cells of the body with the aim of obtaining data about the quality of the red cells detained by the spleen and to repeat the experiments of Pearce. The resistance of the red cells of the spleen pulp was compared with the resistance of the red cells of the body (1) to hypotonic salt solutions, (2) to saponin solutions. At the same time the resistance curves for both kinds of blood, as shown by Brinkman, were determined and compared. Eight experiments were performed in each case. Blood of the general circulation was taken by puncturing a given vessel. Blood from the spleen was obtained by making a cut of 4 to 5 mm. on the surface of the spleen a few millimetres deep. The blood obtained in this way was blood contained in the spleen pulp. It may be mixed to a certain extent with blood which, at the moment of taking, was passing through the spleen, but it was thought that, if a difference should be found between this blood and the blood of the body, the known error would not disturb the conclusions because it would mean that the differences between the possible pure blood of the spleen pulp and

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the blood of the body would be still bigger. It was considered better not to change the normal circulation through the spleen by ligating the artery or the veins. Blood of the splenic vein was not used, many workers have investigated the properties of the blood from this vein and have found extremely variable results. The blood of the splenic vein is the concentrated expression of every change in the volume of the whole spleen. Knowing how easily the spleen takes up and squeezes out blood, it is obvious that differences will easily occur in the qualities of the blood of samples subsequently taken from the splenic vein.

To test the resistance of a sample of blood to a given concentration of salt or saponin solution, the number of cells was stated which remained intact at the certain concentration of the hæmolytic agent, but not the amount of hæmoglobin which has left the cells and has gone into the diluting fluid, as it is usually done. A modification of the method of Liebermann (8) was used. The blood sample was taken with the usual pipettes for counting red cells. The blood was diluted to the mark 101 with a salt solution of a given concentration instead of diluting it with a normal salt solution. As hypotonic salt solutions, sodium chloride solutions were used and the set of different concentrations was prepared by the drop method of Hamburger. With eight pipettes, eight samples of blood were taken simultaneously and every one of them was diluted with one of a set of salt solutions, these latter differing from each other in concentration by 0.04 p.c., the solution of lowest concentration causes a complete hæmolysis, the highest concentration is a normal solution which does not change the cells. In this way there are eight equal samples of blood each diluted with equal quantities of salt solutions varying in concentration, these concentrations are decreasing in an arithmetic progression. The pipettes were shaken well and left for 20 minutes, then shaken again and the cells in each were counted with the Buerker chamber by the usual method for red cells. The percentage of hæmolysis was calculated, using as basis the normal number of cells. It was first found that the process of hæmolysis is completed during this 20 minutes and that no further changes in the number of red cells occurred either when in the pipettes or when brought into the counting chambers. Many preliminary experiments demonstrated this. The number of red cells resistant after 20 minutes to a certain hypotonic concentration of salt does not change in the chamber for hours. This is not the case when saponin solutions are used. The saponin acts more slowly and to obtain a constant number of cells permanently resistant to a given concentration of saponin one has to wait not less than 1 hour. In all experiments

with saponin the count was begun after 1 hour and 20 minutes. The saponin solutions were made as described by Rywosch(4). A saponin standard solution of 1 : 100 of a normal salt solution was prepared and kept sterile. In every experiment from this 1 : 100 saponin solution eight further solutions of 0.01 c.c. to 1 : 3 c.c. to 10,000 of a normal salt solution were prepared with a difference of 0.2 between each of them. The diluted saponin solutions should be used the same day.

There is no difficulty in the counting of the cells which have remained resistant to a certain solution. The cells in the chamber vary slightly in size, the bigger ones have taken up water and probably lost hæmoglobin, the smaller ones have not been changed but the contours of all cells are intact and thus the counting is not disturbed. The cells remain as such until bursting, after which they disappear in the optically heterogeneous mass of fluid which is not visible in the counting chamber. L. E. Bayliss(7), discussing the work of Brinkman on reversible hæmolysis, has found that, with the increase of hypotonicity, the background visible under the microscope is composed mostly of indistinct cells and detritus. He has used slides made of ordinary glass, not chambers of uniform depth, and the red cells observed by him were taken from the paste of a centrifuged blood which was diluted with a hypotonic salt solution equal in amount to the lost serum. Thus in his preparation half of the mass visible under the microscope consisted of cells, whereas in the blood counting chamber the proportion of cells to the whole mass of fluid are in the ratio of 5 to 101, hence the difference in observations.

Results with hypotonic solutions. The experiments were performed on cats. urethane was used as an anæsthetic in all cases and chloroform was given only at the start in a few experiments. The urethane was injected subcutaneously 1 hour before the experiment in doses of 2 c.c. of 25 p.c. solution per kilogram body weight. In preliminary experiments it was ascertained that the resistance of the red cells of the body does not change during the experiment under the influence of the anæsthetic or other possible factor. In some experiments the samples were taken first from the general circulation and then from the spleen and in other experiments the opposite order.

Brinkman(3), investigating the resistance curves of red cells of different animals treated with balanced solutions containing NaCl, NaHCO_3 , HCl, CaCl_2 and CO_2 , obtained curves as shown in Fig 1a. He concluded that in the circulating blood there are clearly expressed three groups of red cells with different resistances against given hypotonic salt

solutions First a group which is most resistant and which forms about 10 p c of all cells, then a group to which the greatest part of the red cells belong, about 80 p c, with a middle resistance, and a third group of cells of very little resistance which are laked at a very low degree of hypotonicity He supposes the first group to consist of young cells, the third of old, and the middle group of the average grown cells As is shown in Fig 1 *b* the resistance curve of the red cells from the general circulation obtained by us is very nearly the same as that of Brinkman The angles of the curve are not so sharp, but the three groups of cells are well distinguished from each other In speaking of groups it must be understood that the term is an artificial one used for convenience, actually there is a complete graduation in Fig 1 from corpuscles which resist a salt solution of 0.64 (or 0.32) to those which resist 0.36 NaCl (or 0.18 balanced solution), but the vast majority are laked by salt solutions of 0.52-0.48 In Fig 1 *c* this graduation is shown better, there

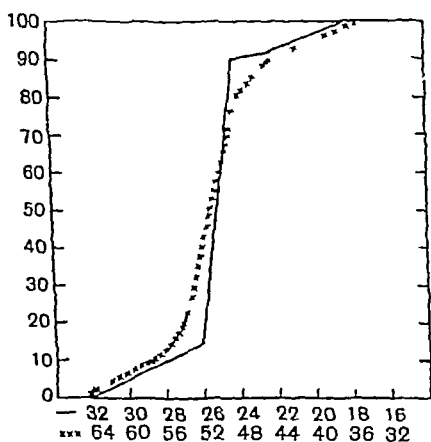


Fig 1 *a* and *b*

Fig 1 *a* and *b* Ordinate Percentage of corpuscles hemolysed.

Abscissa Concentration of solutions

a —, Brinkman. *b* x x x x, NaCl.

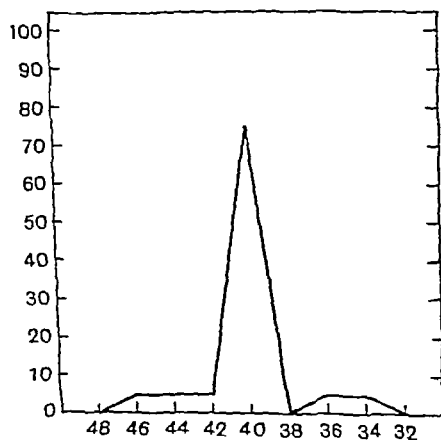


Fig 1 *c*

Fig 1 *c* Percentage concentrations of hemolysis added at each subsequent degree of hypotonicity

the curve represents the amount of hemolysis which is added at each subsequent degree of hypotonicity In all experiments, when the red cells of the blood withdrawn from the general circulation are treated with salt solutions near the isotonic concentration, a certain number of cells are laked with each small decrease in concentration, with greater dilutions, however, there is a considerable range within which decrease

in the concentration does not markedly affect the number of cells which are laked, until a certain concentration is reached at which the majority of cells disappear sharply and there remain a number of cells which resist for a long time further dilution in salt solution

The curve in Fig 1 *b* is an average curve of eight experiments. When this curve is compared with the curve obtained from red cells of the

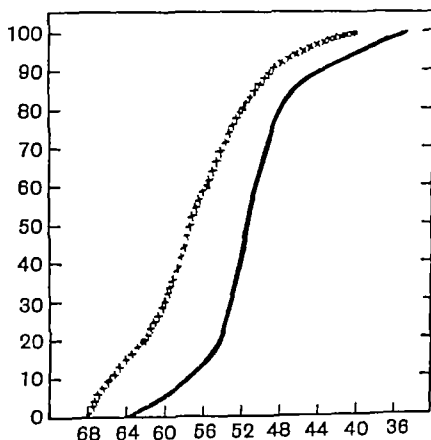


Fig 2a Concentration NaCl in p c.

—, Cells of general circulation. x x x x, Spleen pulp cells

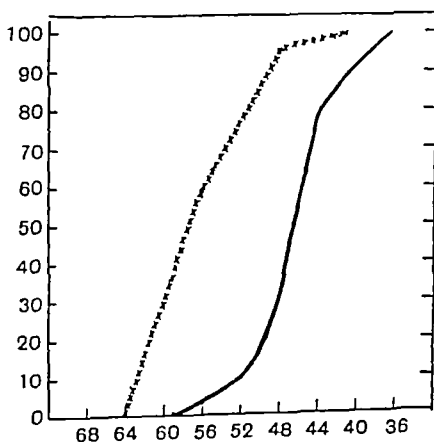


Fig 3

Fig 3 Concentration NaCl in p c

—, Cells of general circulation x x x x, Spleen pulp cells

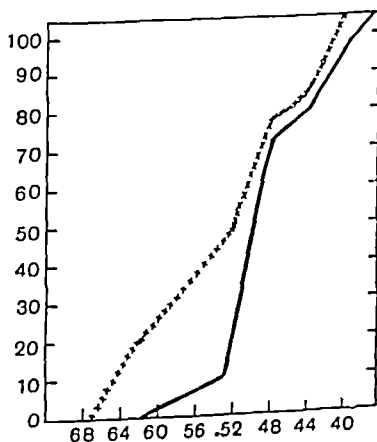


Fig 4

Fig 4 Concentration NaCl in p c

—, Cells of general circulation. x x x x, Spleen pulp cells

spleen pulp taken at the same time and treated with the same salt solutions, a difference in the behaviour of both kinds of cells is obvious. Fig 2*a* represents the two average curves of all experiments, Figs 3 and 4 show two single experiments with the greatest and the smallest differences. There are two points in which the curves differ from each other: (1) the red cells of the spleen pulp are less resistant to hypotonic salt solutions than the red cells of the general circulation, and (2) the curve of the spleen blood shows a different shape. Not all three clearly expressed parts in the resistance curve of the cells from the general circulation exist in the spleen blood curve. The most resistant group of cells is the same in both curves, only less resistant in the spleen blood curve, but the two other groups of cells in the spleen blood curve cannot be distinguished from each other. The curve rises in a straight line and the difference in the resistance between the spleen blood and the blood of the body is the greatest in this part of the curve. The least resistant group in the curve of the general circulation either does not exist in the curve from the blood of the spleen pulp or it is laked at once.

In Fig 2*b* the difference in the resistance between the spleen blood

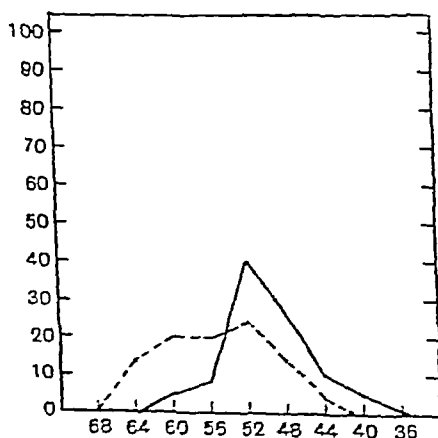


FIG. 2*b* Concentration NaCl in p.c.

— — — Spleen pulp blood. —, Blood from the general circulation.

and the blood of the body is shown in the same way as in Fig 1*c*, the curve shows the amount of haemolysis added at each subsequent degree of hypotonicity, it may be seen that a greater number of cells of the spleen blood pulp are laked at lower degrees of hypotonicity than the number of cells of the body and that the haemolysis begins at a higher salt concentration. The differences in the concentrations of the salt

solutions at corresponding percentages of hæmolysis are given in Table I

TABLE I

Percentage of hæmolysis	0	10	20	30	40	50	60	70	80	90	100
Differences in salt concentrations necessary to cause hæmolysis of same degree for both kinds of blood	0.4	0.9	0.9	0.7	0.6	0.6	0.5	0.4	0.4	0.5	0.5

The conclusion arrived at by comparison of both curves would be that the spleen blood has not only a diminished resistance, but that the spleen pulp blood is of a different mixture of cells. To avoid possible errors the resistance curves were determined in control experiments for the blood of the spleen pulp, the blood from a brachial artery and the brachial vein. The curves obtained were the same as before and there was no difference between the curve for the blood of the artery and of the vein either in regard to the shape of the curve, or the degree of resistance. In other controls blood was taken from muscles by making cuts in the latter, and compared with the blood from arteries and veins of the body, no difference in the resistance was found. Only in the first experiment was there a non-typical result in so far as the spleen blood curve crossed the curve of the blood of the body, nevertheless both curves had the typical shape. Another unexplained result was obtained with the blood from the vena hepatica, an ear vessel and the spleen pulp. The curve of the liver blood had the same shape as the blood from the ear but was less resistant than the latter, lying between the curve of the blood from the ear and that from the spleen pulp.

Results with saponin. The resistance of the red cells of the spleen pulp and the red cells of the general circulation was then tested against saponin solutions. The experiments were performed in the way already described. The results obtained may be seen in the average curve of all experiments shown in Fig 5. The differences in the concentrations of the saponin solutions at corresponding percentages of hæmolysis for both kinds of blood are

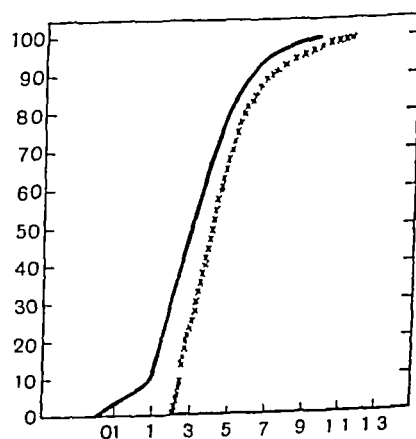


Fig 5 Concentrations of saponin solutions
—, Cells of general circulation
x x x x, Spleen pulp cells

given in Table II. As is seen from these curves the red cells from the spleen pulp compared with the red cells from the general circulation behave towards a given saponin solution in a way opposite

TABLE II.

Percentage of hæmolysis	0	10	20	30	40	50	60	70	80	90	100
Differences in saponin concentrations necessary to cause hæmolysis of same degree for both kinds of blood	0.9	1.1	1.2	1.2	1.1	1.0	0.8	0.6	0.8	1.0	1.6

to that towards a hypotonic solution. They are more resistant to saponin solutions of given concentration than are the red cells from the general circulation. The shape of the resistance curve of the body blood shows the same three groups of cells as the resistance curve to hypotonic salt solutions. It is to be noted that the least resistant group appears smaller, and that the whole curve is steeper. On the other hand, the curve of the spleen pulp cells against saponin, instead of having three well-marked portions, has only two that which corresponds to the corpuscles of lowest resistance, and which tends in the blood curve to become tangential, is absent from the spleen

curve. The end points of both curves are nearer together and the differences in the concentration necessary to cause the same percentage of hæmolysis are smaller. In general both curves are less characteristic, but the main result demonstrates the fact that to saponin the red cells of the spleen pulp are more resistant than the cells from the general circulation. In four experiments a number of samples of both kinds of blood were tested, and other samples taken at the same time were tested against hypotonic salt solutions. The average of the curves obtained are shown in Fig. 6.

Rywośch testing the resistance of the red cells of different animals

to different hæmolytic agents observed the fact that there exists a certain antagonism between the resistance to saponin and the resistance to

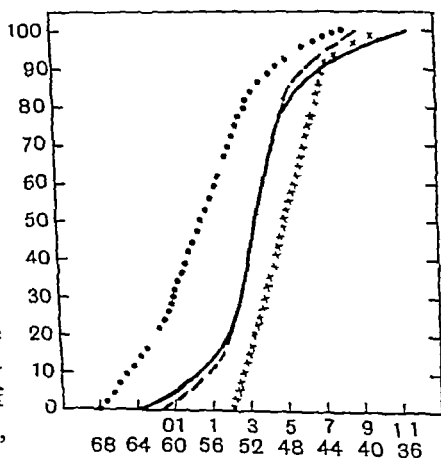


Fig. 6

- , Cells of general circulation against hypotonicity
- - -, Cells of general circulation against saponin.
- , Spleen pulp cells against hypotonicity
- x x x, Spleen pulp cells against saponin

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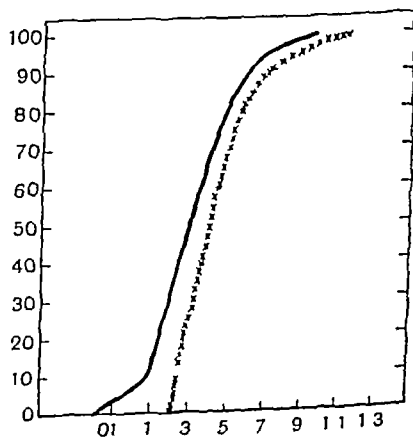


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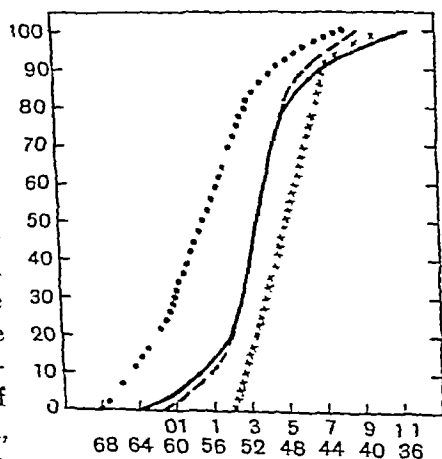


Fig. 6

- , Cells of general circulation against hypotonicity
- - -, Cells of general circulation against saponin.
- x—, Spleen pulp cells against hypotonicity
- , Spleen pulp cells against saponin.

Ry wosch testing the resistance of the red cells of different animals

to different hæmolytic agents observed the fact that there exists a certain antagonism between the resistance to saponin and the resistance to

hypotonic salt solutions If different animals are graded as regards the resistance of their red cells to saponin, the following order would be observed lamb, goat, ox, cat, grey mouse, pig, grey rat, dog, white rat, rabbit and guinea-pig The resistance of their red cells against hypotonic salt solutions arranged them in the following order guinea pig, white rat, dog, grey rat, rabbit, pig, grey mouse, cat, ox, goat and lamb In other words just the reverse of the former grouping Port(5) pointed out that the order of animals as regards the phosphoric acid content of their cells, as given by Abderhalden(6), is similar to the order of resistance to saponin and the reverse of the order of resistance to hypotonic salt solution In other words, the higher the content of a cell of phosphoric acid, the more resistant it is to hypotonic salt solution, and the less resistant it is to saponin Applied to the results of the present experiments, this conception would mean that the difference in the degree of resistance of the cells from the spleen pulp and from the general circulation to hypotonic salt solutions on the one hand, and the reverse relationship of their resistance to saponin solutions on the other hand, could be explained by differences in the salt content of the same kind of cells, especially by differences in their content of phosphoric acid The red cells of the spleen pulp would contain less phosphoric acid than the red cells from the general circulation And the three groups of cells, seen in the curve of the resistance of the cells of the general circulation, would mean differences in the content of phosphoric acid among the cells of the general circulation Brinkman calls them young, old, and the middle group Judging from the present results the "old" cells, the least resistant, are the cells which are most changed by the spleen, because the part of the curve of the blood from the general circulation formed by them disappears in the curve of the spleen pulp cells, but there is a further consideration being the least resistant to hypotonic salt solutions, they would seem to contain the smallest amount of phosphates of any of the cells of the blood of the general circulation, for they are the most resistant to saponin, and in the saponin curve for the cells of the general circulation they occupy the same position as that of the young cells in the curve against hypotonic salt solutions But just this group seems to be the least changed in the spleen pulp red cells' curve, so that according to the results of the present work there are but meagre data upon which to base more than the statement that the red cells which are detained by the spleen are different from the red cells in the general circulation in regard to their content of salts

The question arose What is the cause of this difference? Is the

resistance of the red cells to hypotonic solutions reduced during their residence in the spleen, or alternatively, does the spleen sift from the blood the least resistant element? In the latter case the resistance of the red cells in the spleen pulp would not alter with the length of time they were there. In the former case one might expect that the longer a cell remained in the pulp, the less resistant it would become.

The next experiments were therefore as follows. The resistance of (1) the red cells of the general circulation, and (2) the red cells which had remained some time in the spleen pulp were compared with that of the red cells taken from the spleen pulp immediately after it had refilled itself with blood. In order to get blood which had just entered the spleen, the splenic nerves were uncovered as far as possible from the spleen, prepared and stimulated by an ordinary electrode. With a little experience the nerves may be found and prepared in a few moments. The stimulation causes a large and obvious contraction of the spleen, the spleen loses nearly half of its volume, the previously smooth surface becomes rough, granulated and hard, and the colour changes from bright to dark red. The contraction follows about half a minute after the stimulation. Three to five minutes after the stimulation has ceased the organ slowly refills itself, and the surface regains its smoothness and its former colour. Red cells from the refilled spleen were taken 5 minutes after the stimulation ceased. To avoid damaging the spleen, and so interfering with its power of contraction, only three samples of each kind of blood were taken. These were tested against the same hypotonic solutions and the results are shown in Table III. They show that the red cells which have

TABLE III.

NaCl concentration p.c.	Percentage hæmolysis in blood from		
	General circulation	Spleen before contraction	Spleen pulp after contraction
44	91	100	100
52	56	91	92
56	0	46	44

just been detained by the spleen have the same resistance as the red cells which have been contained in the spleen pulp before the contraction and are less resistant (to the same degree as the latter) than the red cells from the general circulation.

The above experiments do not exclude the possibility that the resistance of the red cells is altered within a few seconds of their arrival in the pulp and undergoes no further change. This, however, seems to be a less likely contingency than that the less resistant cells are those which

are most easily caught either because they are less plastic or for some other reason

In conclusion, it may be worth recording that we have repeated and confirmed the observation of R M Pearce, Krumbhaar and Frazier which originally drew our attention to this subject

The resistance of the red cells of the general circulation of four cats was determined. The next day the cats were splenectomised and the resistance of the red cells of the same vessels—ear—determined at different intervals. The changes in the resistance are given in Fig 7. The resistance increases rapidly for a certain time and then comes back to the previous level. The time of this recovery differs considerably in different animals. The cats stood the operation well, the wounds healing by primary intention.

SUMMARY

1 The red cells contained in the spleen pulp are less resistant to hypotonic salt solutions than the red cells from the general circulation, and the former are more resistant to saponin solutions than the latter

2 The first statement means that it is probable that the red cells detained by the spleen have a different content of phosphoric acid than the red cells of the general circulation

3 The red cells contained in the spleen pulp immediately after the spleen has been emptied and refilled have also a different resistance to hypotonic salt solutions from the red cells of the general circulation

4 The statement of R M Pearce that after splenectomy the resistance of the red cells of the general circulation is increased for a certain time has been confirmed

I am much indebted to Prof Barcroft for the subject of this work, and his aid throughout the research

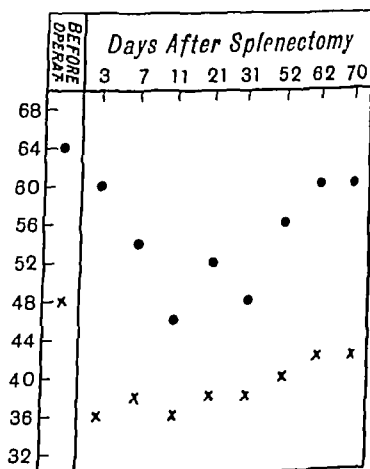


Fig 7 Ordinates Concentration of NaCl in p.c.

●—Beginning of haemolysis.
x—Complete haemolysis

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THE DETERMINATION OF THE HYDROGEN-ION CONCENTRATION OF THE BLOOD

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A Introduction The work reported in this paper had for its object a comparative study of three of the most valuable methods of determining the hydrogen-ion concentration of the blood, and arose from the development of the glass electrode technique (Brown(1), Kerridge(2)), which, from the point of view both of its accuracy and of its convenience in use, seemed an appropriate standard with which the Dale-Evans colorimetric method might be compared, particularly since a certain amount of controversy has arisen as to the accuracy of this last method (for literature see Lepper and Martin(3)). For the sake of completeness, and in order to check any such consistent errors as might arise, (1) from loss of CO_2 , or (ii) from the depolarisation of the hydrogen electrode through reduction of CO_2 by the platinum black, the use of this electrode also was included in the comparison. The results given thus serve to determine both systematic differences (if any such exist) between the three methods, and also their individual instrumental and observational errors, they are an amplification of results already published by two of us(4).

B Technique (1) *Hydrogen electrode* The chief difficulty in making accurate measurements on blood with the hydrogen electrode is the necessity of complete elimination of oxygen, and in this series of experiments the blood was always carboxylated by preliminary saturation with a hydrogen-carbon monoxide mixture. In this way the blood was very easily and completely saturated with hydrogen in the electrode vessel, the measurements with the glass electrode and with the colorimetric method were not upset by drifts due to a slow absorption of oxygen, and the readings with the hydrogen electrode were not rendered inaccurate by a somewhat doubtful correction for the change in reaction with oxygenation.

¹ George Henry Lewes Student.

² Working for the Medical Research Council.

The final saturation with hydrogen and CO_2 was carried out in a tonometer fitted with a platinised platinum electrode which dipped into the blood when it was stood upright the tensions of CO_2 were varied from 20 mm. to 100 mm. The blood was put into communication with a saturated KCl calomel half-cell through the tap of the tonometer by means of a saturated KCl bridge the calomel cell was standardised at the beginning and end of every series of experiments against the potential of the hydrogen electrode in $M/20$ potassium hydrogen phthalate taking the values given by Clark(6) as correct diffusion potentials being considered eliminated by the saturated KCl bridge. The observed potentials were corrected to 1 atmosphere of dry hydrogen. In all cases the readings given are the means of four the tonometer being dismounted and shaken between each pair as a rule all four agreed to within 1 millivolt, but once or twice the potential showed a drift upwards and readings were not taken until a steady state had been reached if there was any sign of a drift downwards the preparation was discarded.

In those experiments in which the hydrogen electrode was not used the carboxylation and the saturation with CO_2 were dispensed with, and the pH varied by addition of acid or alkali to the blood.

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(3) *Colorimetric method.* As in previous comparisons(4) the method used was substantially that described by Dale and Lovatt Evans(5) with a few additional precautions. The dialysing membranes were immersed in weak alkali as well as in 50 p.c. alcohol after testing for leaks with blood, by this means more of the staining matter in the collodion seemed to disappear and this caused a marked improvement in permeability (Taylor(9)) Permeability tests were carried out before every dialysis. Since the blood was in some cases taken from tonometers, it was found convenient (for avoiding loss of CO_2) to run it into the membranes beneath a layer of paraffin. Control readings were taken to ascertain whether the paraffin would alter the readings by clogging the membranes, but no difference could be observed.

In view of the figures published by Lepper and Martin(10) we have

THE DETERMINATION OF THE HYDROGEN-ION CONCENTRATION OF THE BLOOD

BY L E BAYLISS¹, PHYLLIS TOOKEY KERRIDGE²
AND RUTH CONWAY VERNEY²

(From the Department of Physiology and Biochemistry,
University College, London)

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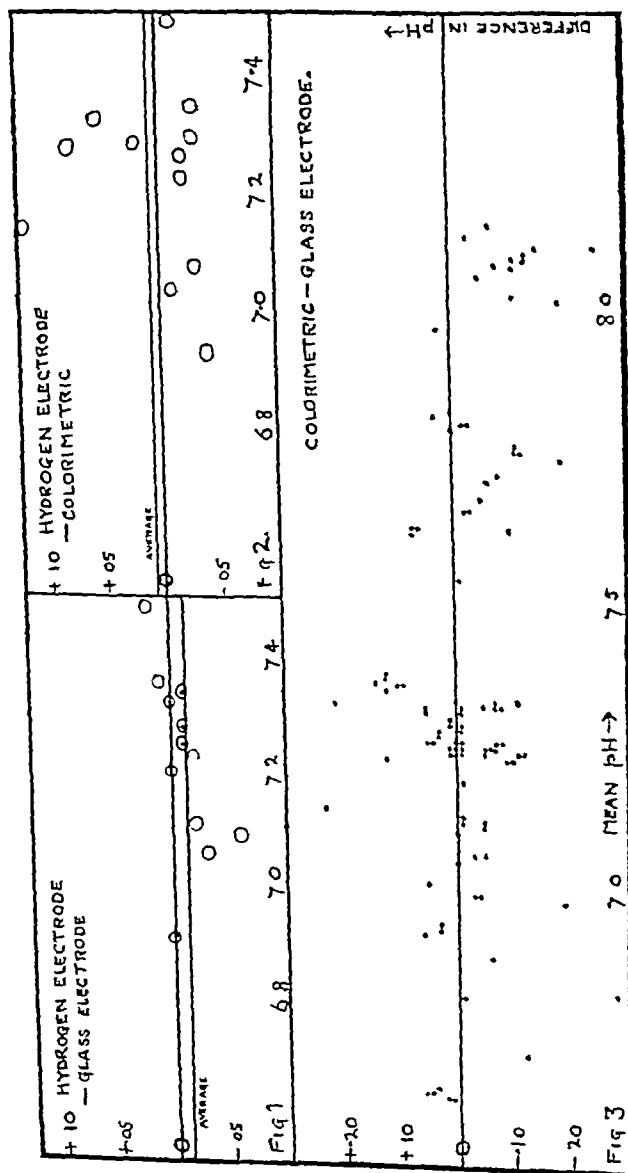
assumed that within the range of pH considered the salt error of the indicator is within the experimental error of the method, since the salt content of the buffer was approximately that of the blood. The sodium phosphate and caustic soda buffer mixtures were $M/15$ of pH 6.5, 7.5 and 10.5 respectively, were standardised by the glass electrode and the hydrogen electrode and were renewed every three weeks.

As a result of the experiments reported by Shaxby and Jones⁽⁸⁾, in which they found that the colour of the indicator—and hence the apparent hydrogen-ion concentration—varied with its concentration even when the depth of the solution was varied inversely, so as to keep the intensity of the light transmitted constant, we thought it advisable to control our observations in this respect. Experimentally, we have been able to confirm Shaxby and Jones quantitatively, in that there is a significant dilution error in the case of neutral red, in the case, however, of brom-phenol, brom-thymol blue, phenol red and thymol blue no change of colour on dilution could be observed, we were unable to agree as to the effect on cresol red. Theoretically, it is easy to show that if the colour observed depends only on the degree of ionisation of the indicator, it must, of necessity, be independent of the dilution, provided that the Law of Mass Action is obeyed. To account for the observed effect with neutral red, we suggest that the tautomeric change of the undissociated indicator (which gives rise to the change in colour) takes place with a change in molecular weight, in which case the colour would not depend solely on the degree of ionisation of one of the tautomers, as it does normally, but also on the total concentration of the indicator. We did not use neutral red in any of our determinations.

In most cases four determinations were made on each sample of blood, and in a series of 96 observations the probable error of a single determination was 0.023 pH , while the probable error of the mean of a group of four was 0.0115. In a series of 45 tests with three readings only on each sample, the probable error of the mean of a group was 0.0112. We recommend, however, that in all cases at least four determinations should be made, if reasonable consistency be desired, since one of the dialysates has not infrequently to be rejected, through leakage or other causes.

C Results and conclusions Our experiments do not give any indication that there is any systematic difference between the values of the hydrogen-ion concentration of blood determined by any of the methods used. This can be seen from Figs 1, 2 and 3. In Fig 1 the difference between the pH of each sample of blood determined by the hydrogen

electrode and by the glass electrode is plotted against the mean pH , and in Figs 2 and 3 are plotted the same values for the hydrogen



Figs 1, 2 and 3 Charts showing distribution of the differences in the readings of the pH of blood given by the hydrogen electrode, the glass electrode and the Dale Evans colorimetric method. Ordinates, differences between the readings on the same sample of blood, abscissae, mean of these readings.

electrode and the colorimetric method, and the colorimetric method and the glass electrode respectively. If there were no instrumental

errors and no systematic differences, all the points would lie on the axis of abscissæ, the fact that they are grouped fairly uniformly on both sides of it shows that if there are any systematic differences, they are within the limits of experimental error

If we take the average difference between the readings by any two of the methods as a measure of the systematic difference between them, we get

$$\begin{aligned} p\text{H (hydrogen electrode)} - p\text{H (glass electrode)} &= -0.011 \\ p\text{H (hydrogen electrode)} - p\text{H (colorimetric)} &= +0.008 \\ p\text{H (colorimetric)} - p\text{H (glass electrode)} &= -0.009 \end{aligned}$$

Now the probable errors of the determinations by the three methods are

Hydrogen electrode	0.003
Glass electrode	0.008
Colorimetric	0.011

expressed as the probable error of the mean of 4, 3 and 4 readings respectively, since these were the number of readings taken on each sample of blood. The probable errors were calculated from the formula $8543 \frac{\sum d}{\sqrt{n(n-1)}} \frac{1}{\sqrt{m}}$, here d denotes the deviation of any particular reading from the mean of the readings on that particular sample of blood, n the total number of observations on all the samples of blood and m the number of observations on any one sample.

The average difference between the determinations by any two methods is never greater than the sum of the probable errors of the determinations by those methods and only in one case is it equal to it. In this case (that of the hydrogen electrode and the glass electrode) a difference of the same order of magnitude and of the same sign could be accounted for by a difference in temperature which escaped correction, since neither outfit was in a thermostat and the temperature of saturation was often a few degrees less than that of the room in which the determinations were made. Since the volume of fluid in the glass electrode was much less than that in the hydrogen electrode, it would warm up more quickly and this would lead to an error if the measurements were made too soon after the saturation. The difference is too small, however, for it to be at all probable that it is real.

As far as accuracy goes, the colorimetric method is definitely worse than either of the electrometric methods, which at present may be relied upon to give about the same accuracy, taking into account the impossibility of getting rid of occasional hydrogen electrode measurements

which have to be rejected on account of errors of unknown origin. The figure for the probable error of the determinations by the hydrogen electrode gives, perhaps, a somewhat false idea of its accuracy, since it occasionally happened that, although the individual observations by it were closely grouped about a mean, that mean was obviously well removed from the true value, as indicated by the other methods and the known conditions under which the particular blood sample was prepared. Although these were rejected, the suspicion remains that there may have been cases in which an error arising from the same or a similar cause, may have been too small to have been obvious though significant, in such cases, the error would be put down to the other methods.

Technically, the glass electrode is by far the easiest to operate, once the apparatus has been set up and adjusted, admittedly the colorimetric method would appear to be the simplest, but it is our experience that extraordinary care must be exercised in the preparation of the materials and in the general conduct of the determinations if the results are to have any reasonable accuracy. As for the hydrogen electrode, it is, in our opinion, to be regarded solely as the fundamental standard, and should be used only when the conditions can be made nearly ideal and after considerable experience. On all counts we have no hesitation in recommending the glass electrode as the only really satisfactory method for the determination of the hydrogen-ion concentration of the blood.

SUMMARY

1 There are no systematic differences between the determinations of the hydrogen-ion concentration of the blood by (a) the hydrogen electrode, (b) the glass electrode, and (c) the Dale-Evans colorimetric method.

2 The accuracies of these three methods have been determined by means of a large number of comparative observations, the probable error of the mean reading on a given sample is (a) for the hydrogen electrode 0.003 pH (mean of 4), (b) for the glass electrode 0.008 pH (mean of 3), and (c) for the colorimetric method 0.011 pH (mean of 4).

We are indebted to Dr Shaxby and Miss Jones for information on their observations of the dilution error, and to Prof. A. V. Hill for his interest, criticism and advice.

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ON THE OUTPUT OF HÆMOGLOBIN AND BLOOD BY THE SPLEEN BY E W H CRUICKSHANK.

(From the Physiological Laboratory, Cambridge)

BARCROFT'S⁽¹⁾ experiments on the protective effect of the spleen suggest that the spleen, contracting in response to stimuli reaching it from the central nervous system by way of the splanchnic nerves, furnishes the body with an increase in red blood cells. It seemed, therefore, of value to determine the hæmoglobin content of the blood expelled from the spleen during contraction and to secure by direct methods further data upon the blood content of the spleen.

Methods The aim was to measure the contraction of the spleen, to collect the blood from the spleen and to estimate its rate of flow during contraction, to determine the hæmoglobin percentage of the blood, and to relate this to the curve of contraction.

In a few preliminary experiments a plethysmograph was used in order to secure records of the change in volume of the spleen, but it was soon found that the manipulation necessary to place the spleen in a plethysmograph, however gently it may be handled, rendered the results valueless, any exposure of the spleen, far more any handling of it, placed it immediately in a condition which could not be regarded as strictly comparable with the normal resting condition of the organ in the unopened abdomen. Anyone who has a surgical experience of the spleen knows how sensitive it is to environmental changes. To free it from its omental anchorage by tying any of its numerous vessels, to expose it, even, is to stimulate it. The plethysmograph was discarded, and in the experiments here recorded the spleen was not exposed at all. The animal was kept warm by means of an electric table, the anæsthetic used was chloralose, 0.1 gm per kilo for a dog, 0.07 gm per kilo for a cat. All the experiments, the results of which are detailed in this paper, were performed on cats.

Operation The animal, dog or cat, is placed on its right side and a longitudinal incision about 10 cm long and about 6 or 7 cm from the mid-dorsal line is made, commencing over the costal margin. When the peritoneum has been cut the rib margin is retracted and a clip placed on the stomach at the posterior edge of the gastro-splenic omentum. Another

clip is placed on the mesentery above the region of the superior mesenteric vein, the latter clip secures the mesentery to the abdominal wall, and thus when traction is made upon these two clips an excellent view of the area for dissection of the splenic artery and vein and the right splanchnic nerve is obtained. Should the dorsal end of the spleen tend to curl forwards into view, a third small clip is used to secure the mesentery to the abdominal wall, and thus the spleen can be kept absolutely under cover throughout the whole experiment, in fact it need never be seen at all.

To obtain a curve of the contraction of the spleen without a plethysmograph the rate of expulsion of blood from the spleen during its contraction must be determined when the circulation is stopped, and here one must remember that the spleen is partly supplied by blood through the right gastro-epiploic artery. By means of blunt dissection the coeliac axis and the junction of the splenic, mesenteric (usually two), and portal veins are cleared (see Fig 1). A cannula is placed in the superior mesenteric vein.

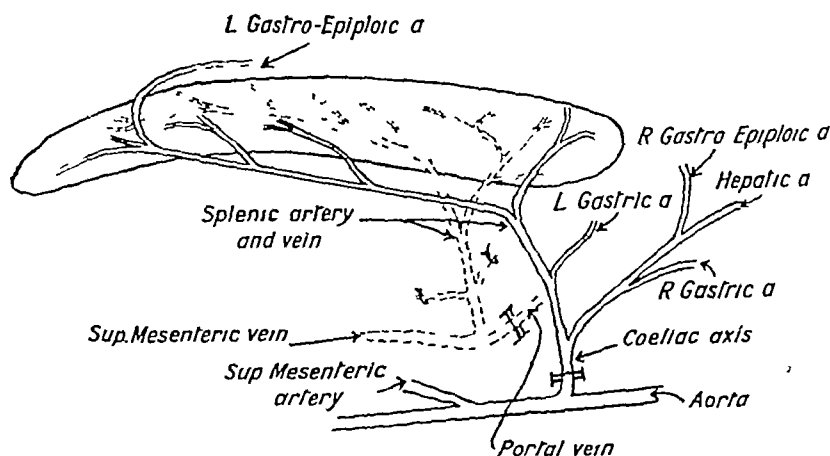


Fig 1 Diagrammatic representation of the splenic and other vessels exposed in the dissection described in the text

A 5 or 10 c c microburette, which has been washed out with saturated potassium oxalate and dried in a current of warm air, is attached at its upper end to the venous cannula, a shielded electrode is placed on the splenic artery, clips are placed in quick succession on the portal vein and coeliac axis¹ and blood allowed free access to the burette. The nerves

¹ This ensures that no collateral circulation will take place by way of the gastro-epiploic branch of the hepatic artery

on the splenic artery are now stimulated with a weak induction current and the rate of expulsion of blood timed on a moving drum with a Jaquet clock marking fifths of a second

I THE HÆMOGLOBIN CONTENT OF SPLENIC BLOOD

Three methods were open for the estimation of hæmoglobin, the hæmoglobinometer, the colorimeter and the determination of the oxygen capacity of the blood. To find which method would give the most consistent results, several comparative estimations were made on cats' blood of varying hæmoglobin strength. Taking the reading for normal arterial blood determined by the Dubosecq colorimeter as 100, the average of the results obtained is as follows

	Haldane hæmoglobinometer	Dubosecq colorimeter	Barcroft manometer
Arterial blood carotid	98.2	100.0	100.0
Venous blood, spleen resting	98.2	100.0	97.5
Venous blood, spleen with splanchnic stimulation	112.8	116.4	117.8
Difference	14.6	16.4	17.8

In using the colorimetric method 1 c c of blood measured from the microburette was added to 1000 c c of distilled water. Since the colorimetric method agreed well with the other two and was least liable to experimental error, it was used throughout. The hæmoglobin percentage varies markedly in different animals, and for the sake of facility in reading all hæmoglobin percentages have been given in terms of the normal standard of 100.

This first series of experiments, both on cats and dogs, was carried out primarily to determine to what extent the spleen may act as a reservoir for hæmoglobin, not only a reservoir for blood, and incidentally thereby able to add to the hæmoglobin content of the body, but a reservoir in the sense that the blood stored in the spleen has a hæmoglobin content in excess of the normal. It was argued that if the spleen be a reservoir for hæmoglobin, then it should be possible to show an increase in the hæmoglobin content of the blood expelled from the spleen during contraction. In the first four experiments upon cats the splenic artery was not clamped, the normal rate of flow was first determined, and then the rate of flow during and after stimulation of the branches of the left splanchnic nerve lying upon the splenic artery. The blood was collected in the microburette, the rate of flow for each successive c c being recorded by a signal, it was then run into distilled water contained in a series of six one-litre graduated flasks, 1 c c being placed

in each flask, and the hæmoglobin percentage determined colorimetrically. From Table I, which gives the details per c c of three experiments, it

TABLE I. Showing relation of the percentage hæmoglobin content of the splenic blood (carotid blood = 100 p c) upon contraction of the spleen, to changes in the output of blood.

		Exp 1		Exp 2		Exp 3	
Splenic blood venous		Hb p c	Output in c c per min	Hb p c	Output in c c per min	Hb p c	Output in c c per min
(a) Before stimulation		—	15 0	—	16 0	—	18 0
{	1st c c	106 9	18 0	102 0	22 0	107 0	21 4
	2nd „	113 0	24 1	116 0	34 0	114 0	37 5
	3rd „	135 7	21 0	134 0	35 0	128 0	39 7
	4th „	114 7	19 5	117 0	32 0	134 0	30 0
(b) During stimulation of splanchnic nerves		93 0	16 0	109 8	26 6	124 0	27 0
{	5th „	—	—	103 5	21 5	—	22 0
	6th „	—	—	—	—	108 0	16 0
	7th „	—	—	—	—	96 0	6 0
	8th „	—	—	—	—	—	—

will be seen that the rate of blood flow through the spleen increases rapidly upon stimulation of the splanchnic branches surrounding the splenic artery. This increase is maximal between the second and fourth c c, after which it steadily declines, and when vaso-constriction is maximal the rate of flow becomes markedly less than the normal. According to Barcroft's work the withdrawal of 10 c c of blood causes little or no alteration in the volume of blood in the general circulation, despite the fact that this amount constitutes a fairly large loss of blood for a cat of 2-3 kilos. In Exp 3 it will be seen that at the eighth c c the blood velocity is reduced to one-third of the normal. If then the spleen is to expel its stored hæmoglobin one would expect it to do so long before its blood supply had become restricted, in fact one would expect that the period of increased output, aided by contraction of the spleen, would show the greatest increase in hæmoglobin. A glance at Table I and Fig 2 shows such a surmise to be correct, and shows also that the greatest output of hæmoglobin takes place with the third and fourth c c.

In order to form a conception of the extent and rate of contraction of the spleen, one must determine the rate of expulsion during a period in which no blood is allowed to enter the organ from the circulation. To measure the rate of flow the portal vein and coeliac axis are clamped as nearly as possible at the same moment, the splanchnic nerve stimulated and the blood collected as previously described. Here, as in previous experiments, the relation of blood flow, or, as in this case, the rate of contraction of the spleen to the hæmoglobin output is clear, the hæmo

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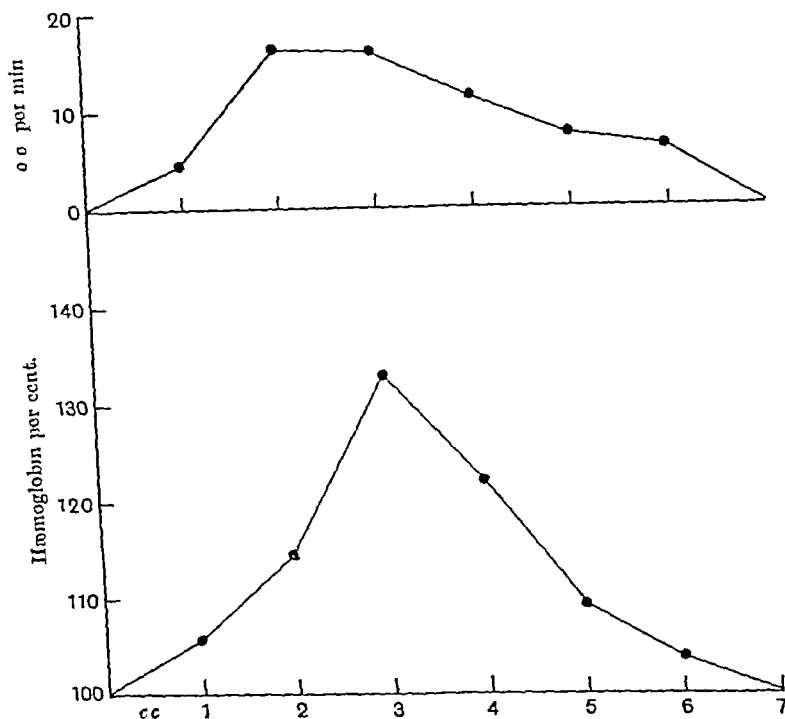


Fig 2. Lower curve, percentage hæmoglobin in successive c.c. output from the spleen on splanchnic stimulation, for comparison with upper curve showing simultaneous increase in rate of blood flow. Average of results in Table I.

shown in Table II and Fig 3. Here again the greatest concentration of hæmoglobin is associated with the third and fourth c.c. of blood and shows an increase which may amount to 40 p.c. (Exp 1, Table II).

Many comparative estimations have been made on the number of red blood cells in the splenic artery and vein. Several workers have found a diminution of red cells in the venous blood of the spleen, others an increase. Paton, Gulland and Fowler(2) came to the conclusion that there was no difference. On compression of the spleen they stated that the number of the red cells was decreased, but the method used for expelling blood from this organ was a very abnormal one. From the results which we have obtained one must conclude that the contraction

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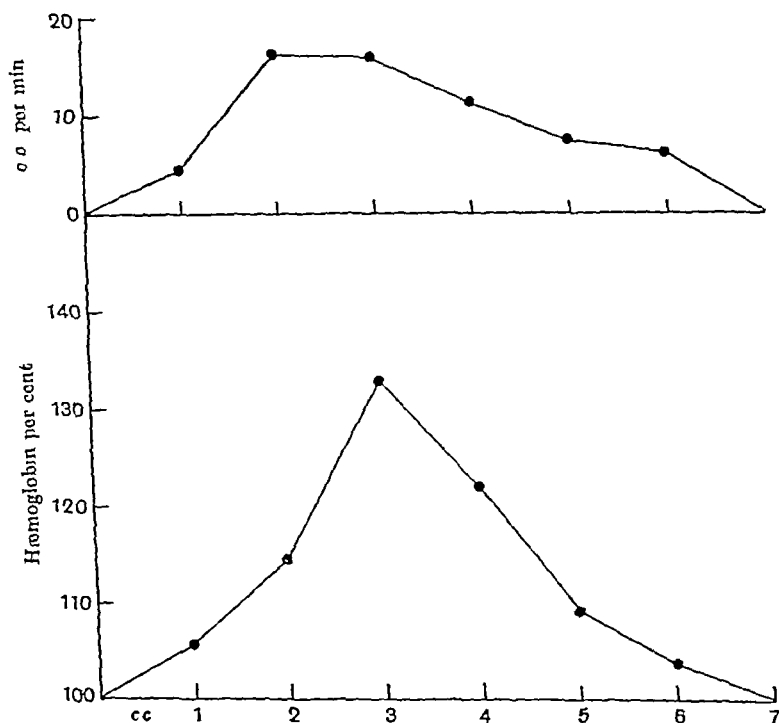


Fig. 2. Lower curve percentage hæmoglobin in successive c.c. output from the spleen on splanchnic stimulation, for comparison with upper curve showing simultaneous increase in rate of blood flow. Average of results in Table I.

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of the spleen does produce an increase in the number of red blood cells, that is, it increases the hæmoglobin percentage of the blood

TABLE II Showing relation of the percentage hæmoglobin content of the splenic blood (carotid blood = 100 p c) upon contraction of the spleen, to the rate of contraction, as shown by the velocity of the blood expelled, during cessation of the circulation in the spleen

Splenic blood venous		Exp 1		Exp 2		Exp 3	
		Hb p c	Output in c.c per min.	Hb p c	Output in c.c per min.	Hb p c	Output in c.c per min.
(a) Before stimulation		—	0	—	0	—	0
(b) During stimulation of splanchnic nerves	{ 1st c.c	104.0	2.6	98.4	2.7	107.0	2.0
	{ 2nd „	122.0	4.7	110.4	4.0	117.2	3.2
	{ 3rd „	138.9	4.7	120.0	3.4	130.0	3.0
	{ 4th „	124.8	3.3	116.8	2.5	115.0	1.8
	{ 5th „	110.0	1.5	—	—	—	—

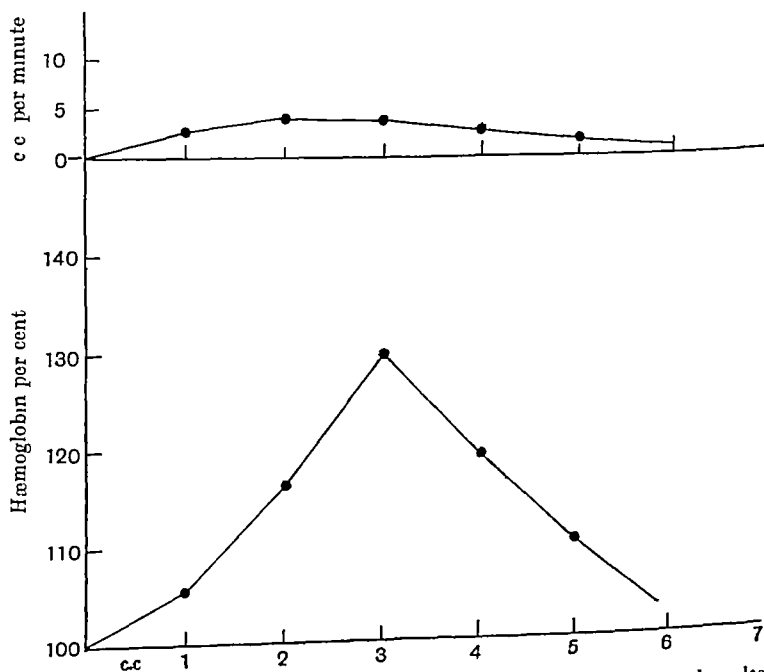


Fig 3 Lower curve, percentage hæmoglobin in successive c.c. output from the spleen on splanchnic stimulation for comparison with upper curve showing simultaneous change in volume of the spleen, the circulation being stopped. Average of results in Table II

II THE SPLEEN AS A RESERVOIR FOR BLOOD

De Boer and Carroll(3), by the plethysmographic method showed that in an animal breathing weak CO mixtures (0.92 p c) the spleen con

tracted 2.3 c.c. in 26 minutes, while with stronger CO mixtures (3.0 p.c.) it contracted in volume to the same extent in approximately 3 minutes. Barcroft has clearly shown that the contraction of the spleen in the body is of an order much greater than that shown by the plethysmograph. The results of the experiments just quoted and those shown in Table III.

TABLE III. Showing time contraction of the spleen and the output of blood upon stimulation of the splanchnic nerves accompanying the splenic artery

Amount of blood expelled in c.c.	1	2	3	4	5	6	7	8	9	10	11	12	13
Exp 1 Time (secs.)	23	36	49	67	97	120	170	217	290	—	—	—	—
Exp 2 Time (secs.)	22	42	59	70	96	136	158	184	229	288	—	—	468
Av Time (secs.)	22.5	39	54.5	71.5	96.5	128	164	200.5	259.5	—	—	—	—

bear this out. Two experiments were carried out in which, under very weak faradic stimulation of the nerves accompanying the splenic artery, the spleen was allowed to contract until no more blood was expressed. A total of 9.5 c.c. and of 13.5 c.c. of blood was obtained in cats weighing 3 and 3.2 kilos respectively. The time for the expulsion of blood per c.c. is seen in Table III. The average of these results up to the ninth c.c. is plotted as a curve of contraction in Fig. 4. The curve in Fig. 3, which represents graphically the rate of output of the blood with the splenic

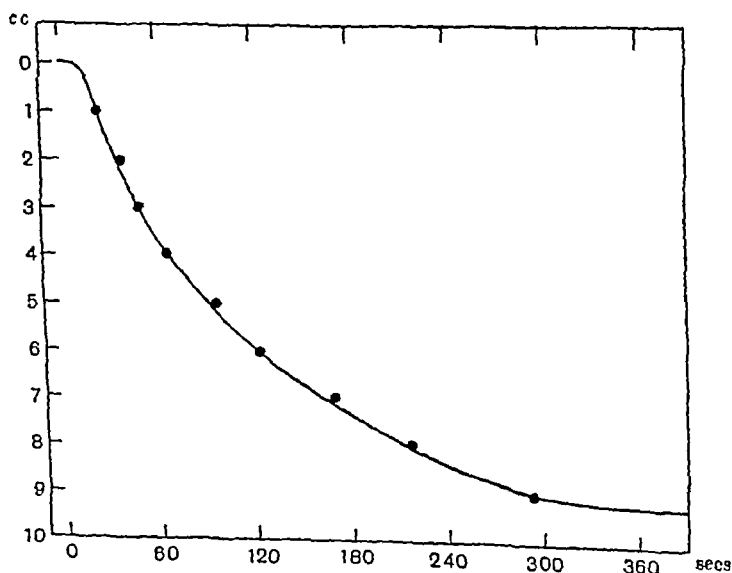


Fig. 4. Curve of contraction of the spleen upon splanchnic stimulation from data in Exp. 1, Table III.

artery clamped, indicates the rate of contraction in c c per minute Fig 4, however, indicates much more clearly the nature of the change in volume It is rather striking that in both these cases (Table III) the first 5 c c should be expelled in 16 minutes in the first case, 9 c c were expelled in 48 minutes, while in the second, 9 c c required only 38 minutes In this latter case 135 c c of blood were forced from the spleen in 78 minutes In these cases, as in the others quoted, the period of greatest activity, namely, during the second to fifth c c, is the period of greatest hæmoglobin output

Discussion of results Since the measurements of the rate of contraction are made on an organ which has been deprived of its oxygen supply for 5-7 minutes, these experiments give no information as to the normal rate of contraction with the circulation intact Further, from such curves as are here given, no one would conclude that the contraction of the spleen is necessarily a uniform movement in the reduction of its volume These results simply indicate that upon splanchnic stimulation the spleen, in cats of 2-3 kilos, will deliver up an amount of blood varying from 7 to 135 c c In this last case the amount of blood produced by the spleen was exactly twice the post-mortem weight of the spleen in grams, a fact which is of interest in view of Barcroft's statement(1) that "the post-mortem weight of the spleen may be taken as a minimum estimate of the volume of blood it drives into the circulation during strong exercises" The mechanism of this function has not, however, been demonstrated It may be that the steady tonic contraction may be a basal movement having superposed upon it rhythmic movements of contraction and dilatation, the former dominating the latter Roy(4) demonstrated normal rhythmic movements in the spleen and Schafer and Moore(5) showed that in asphyxia these normal rhythmic movements are markedly increased

As to the power of the spleen to act as a reservoir of hæmoglobin, the present work would indicate that the hæmoglobin concentration, which undoubtedly takes place there, is not of any considerable significance For example, if, as is possible under the present method of experimentation, 8 c c of splenic blood contained hæmoglobin percentages of 105, 125, 140, 130, 120, 115, 110 and 105 respectively per c c, the amount of hæmoglobin represented by these 8 c c of splenic blood when added to the circulation would be equivalent to an addition of 95 c c of normal blood As an isolated action this is meaningless as a possible continuous rhythmical activity related to all the functional activities of the organism it may not be without some significance

In discussing this action of the spleen in concentrating hæmoglobin we are faced with the possibility that the organs rich in muscle tissue may concentrate hæmoglobin to a certain extent as a result of their activity, the loss of fluid taking place in the capillaries of the resting organ, and being increased by the contraction of the musculature which would force more fluid into the tissue and lymph spaces. We may ask therefore to what extent the slow blood flow in the non-functioning capillaries and the subsequent contraction of a skeletal muscle would tend to concentrate the blood. If it were found that any muscular organ concentrated blood to an extent comparable with the spleen, then the hæmolymphatic structure of the spleen could not be regarded as peculiarly a reservoir of hæmoglobin. The tongue offered not only a suitable muscular organ, but one with its motor and sympathetic nerve supply differentiated, it was therefore possible to determine the effect of muscle contraction uncomplicated by vaso-constriction. Two experiments were carried out in which both lingual arteries and one vein were clamped, and the hypoglossal nerve stimulated with a weak induction current. The results shown in Table IV were negative, indicating that hæmoglobin

TABLE IV Showing relation of the hæmoglobin content of the blood (carotid blood = 100 p.c.) upon contraction of the tongue of the dog, to changes in the rate of output of blood upon stimulation of the hypoglossal nerve

Venous blood from the tongue	Exp 1		Exp 2	
	Hb p.c.	Output in c.c. per min.	Hb p.c.	Output in c.c. per min.
(a) Before stimulation	—	3.0	—	4.4
(b) During stimulation	103.0	3.2	96.8	4.6
of the 12th nerve	105.0	3.4	98.5	5.1
1st c.c.	103.5	3.2	103.0	5.0
2nd "	86.0	2.7	93.5	4.2
3rd "				
4th "				

concentration is not a consequence of muscle contraction. A slight rise in hæmoglobin percentage may be expected where, under pressure, fluid is forced slowly from the blood into tissue and lymph spaces.

SUMMARY

1 A method is described for estimating, in cats, the blood and hæmoglobin output of the spleen without exposure of the organ.

2 The fluid expelled from the spleen pulp is often richer in hæmoglobin than the blood of the general circulation. The increase of concentration is maximal, in cats, with the third c.c. expelled, amounting to from 20 to 40 p.c. of the normal.

3 Corroboration is given to the fact that the spleen is a reservoir for blood

4 The amount of blood which can be added to the general circulation by contraction of the spleen upon stimulation of the splanchnic nerves is of the order of 2.6–5.6 p.c. of the total blood volume of the animal, the amount so added may be equal to twice the post-mortem weight of the spleen in grams

5 The rate of contraction of the spleen upon splanchnic stimulation is shown to be maximal during the expulsion of the first 5 c.c., which represents approximately one-half of the total amount expelled, and takes about 1.5 minutes. It then decreases rapidly, maximal contraction being reached in 5–8 minutes, depending upon the amount of blood expelled

6 It has been shown that the capacity of acting as a reservoir for blood and of concentrating hæmoglobin is a peculiar property of the spleen not shared by other muscular organs, *e.g.* the tongue

I should like here to express my indebtedness to Prof. Barcroft for introducing me to the work on the spleen, and to Prof. A. V. Hill for allowing me facilities for carrying out certain of these experiments in the Department of Physiology, University College, London

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THE IMPULSES PRODUCED BY SENSORY NERVE
ENDINGS Part 3 Impulses set up by Touch and Pressure
BY E D ADRIAN AND YNGVE ZOTTERMAN¹

(From the Physiological Laboratory, Cambridge)

IN Part I of the present series one of us⁽¹⁾ described a method of recording nerve action currents by means of a capillary electrometer and a three-stage amplifier, together with some preliminary observations² on the impulses set up in various types of sensory nerve fibres by stimulation of their end organs. In Part II⁽²⁾ we gave a more detailed analysis of the sensory impulses produced by stretching a muscle, and we were able to show that in a single nerve fibre the impulses usually recurred in a regular series with a frequency depending on the intensity of the stimulus, that the impulses (or rather their action currents) were all of the same intensity and that their frequency was low enough to leave the nerve fibre time for complete recovery between one impulse and the next. As these observations were made on the frog and were confined to one type of sensory ending, we were anxious to extend them to mammals and to some other form of sensation.

The results given in Part I had shown that a cutaneous afferent nerve in the cat (the internal saphenous) usually exhibits a series of action currents and that these increase in number when the skin is pricked or pinched. There is, however, a considerable drawback to the use of such forms of stimulation, since their intensity is not readily measured, and to overcome this difficulty we decided to use moderate pressure as the stimulus in the present research. The end organs sensitive to pressure are not known with certainty, but they are generally supposed to be the touch corpuscles in the skin and the Pacinian and other types of corpuscle in the subcutaneous tissues. Since the latter occur singly or in small groups in the mesentery of the cat, we thought at first that the most suitable preparation would be a single Pacinian corpuscle from the mesentery with its nerve fibre isolated and connected to the electrometer. Unfortunately, we found that various technical difficulties stood in our way. In the living animal it is extremely difficult to detect the

¹ Travelling Fellow of the Rockefeller Foundation.

course of the nerve fibre which runs to a single corpuscle and the larger nerves usually run with blood-vessels and lymphatics in a band of adipose tissue. Damage to the blood supply might seriously interfere with the functions of the corpuscle and we were rarely successful in producing a preparation in which we felt that both the corpuscle and the nerve could be regarded as normal. In three preparations we were able to record undoubted action currents in the nerve, in two of these the nerve came from a group of corpuscles (three in one experiment and eight in the other), but an irregular series of action currents was present without any stimulation and we could not satisfy ourselves that steady pressure (2-5 grm) on a small glass plate resting on the corpuscles made any difference to the number of impulses. The highest recorded frequency (with stimulation) was 95 per sec. In the third experiment the nerve was derived from two corpuscles which were stimulated by touching with a glass rod just before the plate was exposed. With stimulation the highest frequency (during a period of 16 sec) was just 100 per sec and no impulses appeared in the absence of stimulation. Although the difficulties in the way of making a good preparation did not seem insuperable, we felt that it would be wiser to leave the organs in the mesentery and to investigate the effects of pressure in some region where organs sensitive to pressure are known to exist and where the sensory nerves are more easily dissected.

Preparation The region ultimately chosen was the plantar surface of the cat's hind foot. The arrangement of the plantar nerves is shown in Fig 1 (taken from Langley's diagram, this Journ 57 p 434 1923), and it will be seen that the cushion of the 2nd digit is supplied by the slender medial branch of the internal plantar nerve. It contains from 200-400 fibres and is easily dissected out for a length of 1 cm or more without interfering with the blood supply. Most of our observations were made on this nerve, though we have sometimes used the other digital nerves. In a few experiments we used cats anaesthetised with urethane but the majority were made with the spinal animal decapitated under chloroform anaesthesia and allowed to remain an hour or more for the effect of the anaesthetic to wear off. The results with urethane narcosis do not differ appreciably from those with the spinal preparation. When the latter was used the hind limbs were immobilised by nerve section.

The animal lies on its belly on an insulated stand inside the iron-walled box which acts as a shield from electromagnetic disturbances (see Part I). The left hind foot is fixed with the plantar surface upper-

most on a small platform which carries the electrodes. These are of the Ag, AgCl, NaCl gelatin type and end in small camel-hair brushes curved at the tip so that the nerve can rest on them. The nerve is cut just before its junction with the main trunk of the internal plantar nerve and placed on the electrodes with its proximal end slung by a silk thread from an

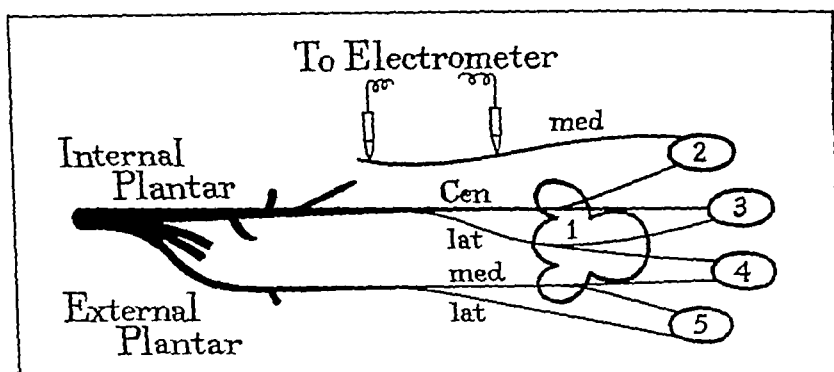


Fig 1 Digital nerves to plantar surface of cat's hind foot

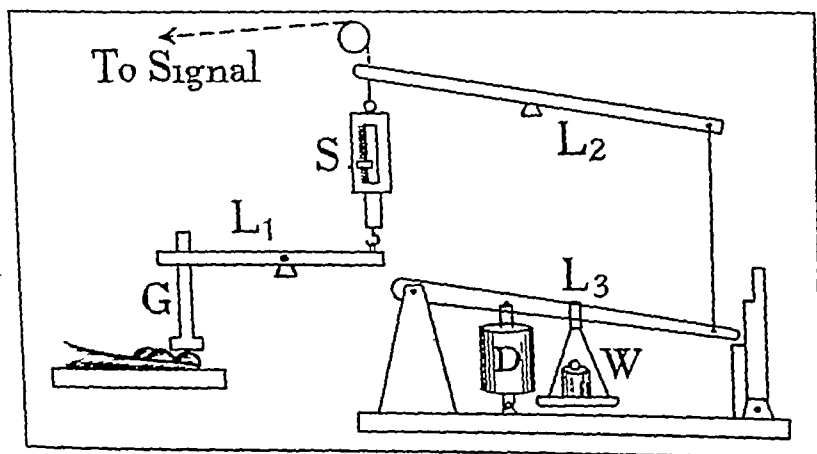


Fig 2 Stimulating apparatus.

insulated support. The nerve is constantly irrigated with warm Ringer and the temperature of the foot is maintained by a small carbon lamp placed a few inches away. The lamp is disconnected before an observation is made as it may be a source of artefacts in the amplifier circuit. The stimulating apparatus consists of a glass rod *G* (Fig 2) ending in a disc 1.1 cm in diameter which is pressed downwards against the

cushion of the pad by a lever L_1 . The lever is pivoted at its centre and the far end is attached to the hook of a spring balance S , the body of which can be pulled up through varying distances by the movement of the lever L_2 . L_2 is connected to a third lever L_3 which is moved downwards at a uniform speed by a weight W acting against the dashpot D . The rate of movement can be varied by altering the weight, the final value of the pull exerted on L_1 is varied by adjusting the length of the arms of L_2 . A light thread attached to L_2 operates a signal lever which moves across the slit of the cinematograph camera and records the increase of pressure on the film.

With this arrangement it is possible to apply a gradually increasing or a steady pressure of any value from 25 to 1500 grm on the pad under the disc G , and to record the pressure simultaneously with the electric responses in the nerve.

RESULTS

(1) *Touch*

The contact of the glass disc with the cushion of the toe will presumably stimulate the receptors in the skin which are sensitive to touch and as the pressure increases the receptors in the subcutaneous tissues will be brought into action as well. If there were a continued discharge of impulses from the receptors in the skin as long as the contact was maintained, it might be difficult to measure the added effect of an increase of pressure. Fortunately, the organs concerned all show the phenomenon of adaptation in a high degree. In some preparations with the medial branch of the internal plantar nerve there is a continuous discharge of impulses at a frequency of 20–30 per sec in the absence of any kind of stimulation, such a discharge is often met with during the first few minutes after the preparation has been set up, but it rarely persists at this rate for more than 5 minutes, and in most preparations the “resting” discharge is not greater than 5–10 per sec. This discharge is not increased under the mere continued exercise of the light contact of the glass disc with the pad. If, however, a record is made as the disc is lowered gently on to the pad a sudden outburst of impulses is seen at the moment of contact lasting for about $1/5$ sec. Fig 3 shows records of this kind. The disc was lowered gently at a speed of 1 cm per sec by a lever controlled by an oil dashpot and the weight finally resting on the pad was 5 grm. In the lowest record the signal is moved by hand at what was judged to be the moment of actual contact. For about $1/10$ sec

the impulses recur so rapidly that there is considerable interference, but within $1/5$ sec the discharge has nearly subsided. Evidently the organs

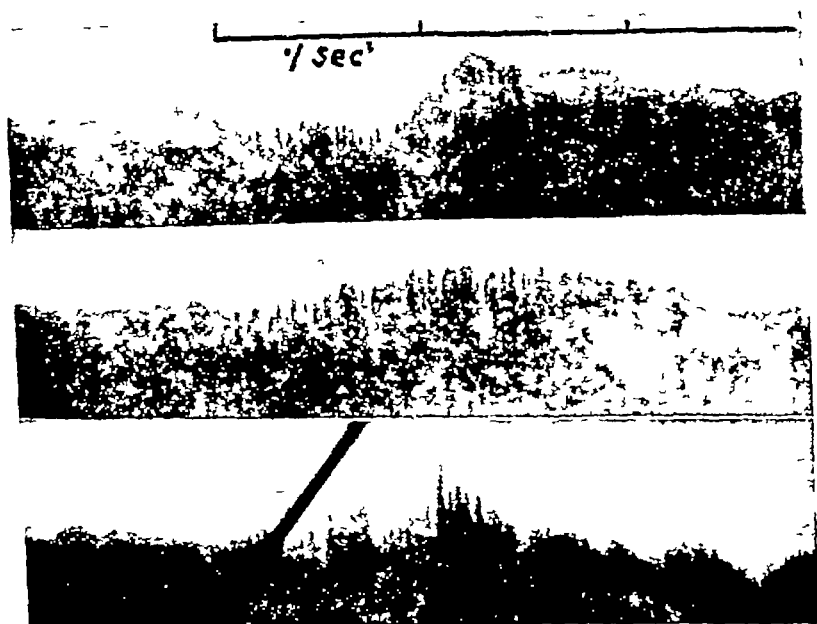


Fig 3 Film records (enlarged) of impulses due to "Touch." Disc lowered gently on to pad and allowed to remain there. In the middle record the weight resting on the pad was 15 gm. In the others 5 gm. Discharge lasts for 1 to 2 sec. at the moment of contact and then subsides

for touch become adapted so rapidly to the stimulus that it becomes almost ineffective in $1/5$ sec. We cannot therefore assume that the impulses set up by moderate pressure are all derived from the subcutaneous organs, for an increase in pressure might again excite the organs in the skin, but, as will be seen later, the low frequencies obtained with the pressure stimulus make it unlikely that more than a few end organs are excited.

(2) Pressure

Nature of impulses In all these experiments the preparation is said to be unstimulated when the disc is resting lightly (weight = 5 gm) on the pad. It is stimulated by releasing the lever L_3 so that the spring balance is extended and the pressure on the pad increased. The rate of movement could be adjusted so that the full pressure was reached in from $\frac{1}{2}$ to 6 sec. The full pressure was generally made equal to 100 gm.

(26 grm per sq mm), 250 grm and 500 grm and records were made as the pressure was increasing and after it had reached its full value. In every case the discharge of impulses reached its maximum frequency whilst the pressure was increasing and then declined rapidly although the pressure was maintained. The actual frequencies of discharge varied over a wide range from one preparation to another but, fortunately for our purpose, the frequencies were usually low enough to allow the individual impulses to appear in the record. The form of the electric responses as they appear in the electrometer record may be seen

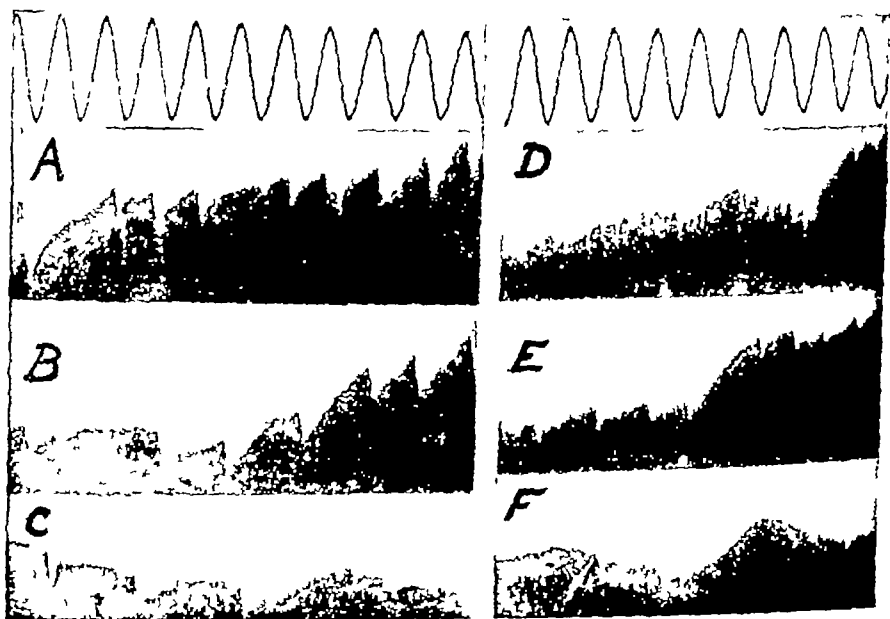


Fig 4. Plate records of impulses due to pressure. Time marker gives 1/100 sec periods.

A, B, C Exp 1

A Slow increase of pressure (100 grm. per sec.) Actual pressure about 350 grm

B Steady pressure of 500 grm for 5 sec.

C Steady pressure of 100 grm for 3 sec.

D, E, F Exp 9

D Slow increase of pressure (100 grm. per sec.) Actual pressure about 450 grm

E Steady pressure of 500 grm. for 5 sec.

F At rest. Disc touching pad.

from Fig 4. In *A, B* and *C* (Exp 1) the responses are mostly monophasic, in *D, E* and *F* (Exp 9) they are mostly diphasic. As the nerve was irrigated with Ringer between each record the magnitudes of the

responses are not strictly comparable but there is evidently no marked variation in size. As in the case of the afferent impulses from the frog's muscle, we have found no indication that a change in the intensity of the stimulus has any effect on the size of the impulses set up, the change is expressed entirely by the altered frequency of the discharge. This point is shown more clearly in Exp 2 where pressures of 50, 100, 250 and 500 grm were applied for 5 seconds. The frequencies corresponding to these pressures are < 15, 57, 140 and 170 per sec, and an analysis of a small group of consecutive impulses from each record is given in

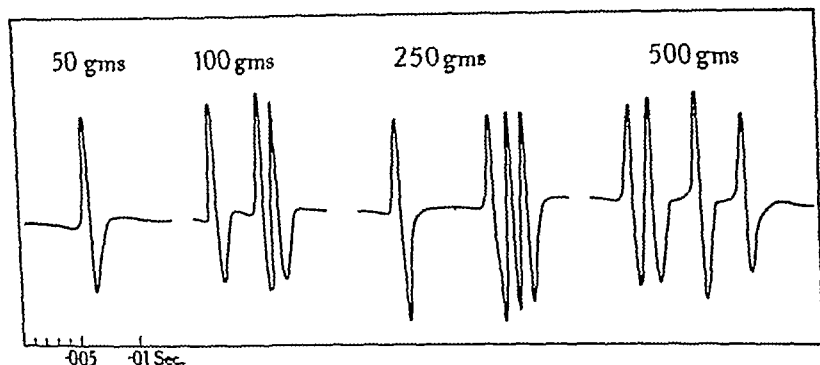


Fig 5 Analysis of electrometer records, Exp 2, showing that the size of individual impulses does not vary with the stimulus.

Fig 5 Since the impulses are not all derived from the same nerve fibre there is a certain amount of variation in size in each record, but the variation is substantially the same in all three records, and apart from the frequency it would be impossible to say which group of impulses is due to the strong stimulus and which to the weak. We conclude, then, that the all-or-nothing relation between the stimulus and the impulse set up holds good for the mammalian pressure receptor just as it does for the stretch receptors in the frog's muscle.

Relation between strength of stimulus and frequency of discharge Owing to the very rapid adaptation which occurs in the end organs, a comparison of the frequencies produced by different stimuli is only valid if the pressure is allowed to act for the same time in each case. Fig 6 gives the frequencies for various pressures applied for 5 seconds in four experiments. The form of the curve is much the same in each case though the frequency for a load of 500 grm varies from 50 per sec in Exp 5 to 305 per sec in Exp 4.

The curves relating frequency to stimulus for a single end organ

would not necessarily have the same form, since the stronger stimuli may bring more end organs into action. It may seem remarkable that

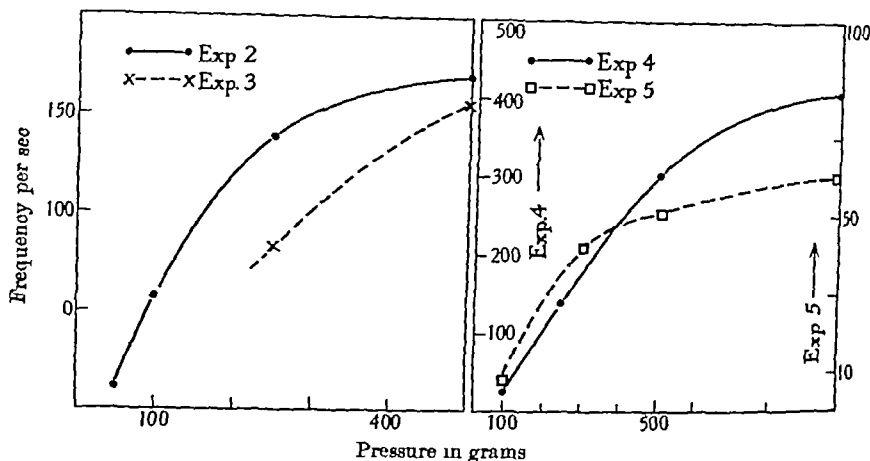


Fig 6 Relation between stimulus and frequency of discharge
Pressure constant for 5 sec. Every impulse counted.

a load of 1000 gm does not produce a much greater increase in frequency, for it might be expected to evoke responses from the pain receptors as well as from those of pressure. But considerable pressure may be applied to the cushion of a cat's toe without causing any signs of discomfort and the pad of the human toe is equally insensitive. In one experiment where a load of 2 kilos was applied the curve showed a great rise in frequency between 500 gm and 2 kilos, and in one a sudden rise occurred between 300 and 500 gm. In both experiments the sudden increase was presumably due to the stimulation of a fresh set of nerve endings.

Adaptation In the tension receptors of the frog's muscle it was found that if the stimulus was maintained at a constant value, the frequency of discharge fell to half its maximal value in about 10 seconds. In the pressure receptors of the cat's toe the rate of adaptation is much more rapid. This may be seen from the two cinematograph film records given in Fig 7. In one of these (Exp 4) the frequency of discharge is always high, and whilst the pressure is increasing the different impulses overlap and produce larger excursions in the electrometer. In the other (Exp 1) the pressure increases more slowly, the frequency is always lower and there is very little overlapping. In both the frequency reaches its maximum almost as soon as the pressure has begun to increase, and it

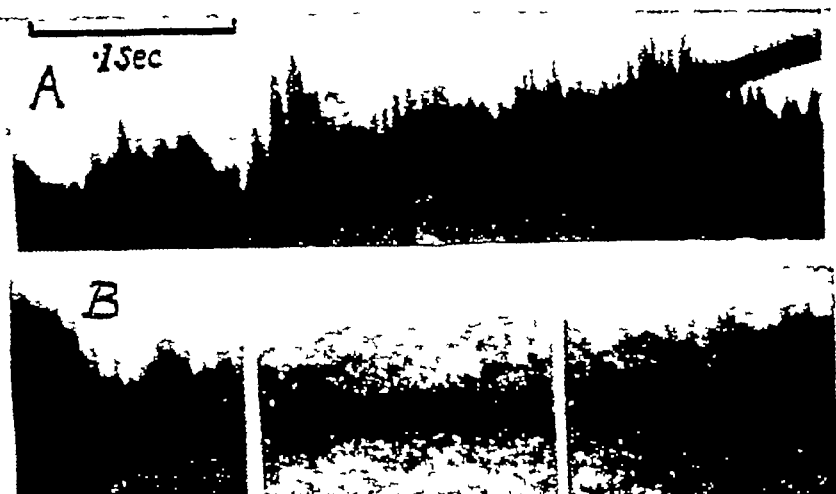


Fig. 7 Film records of response to increasing pressure.

A Exp. 4. Rapid loading (500 gm. in $\frac{1}{2}$ sec.) Interference of impulses as loading begins. Frequency has dropped before maximum pressure is reached. Black line signals increase of pressure.

B Exp. 1. Slower rise of pressure (500 gm. in 5 sec.) Loading begins in first section, midway in second, maximal in third. Frequency greatest at the beginning

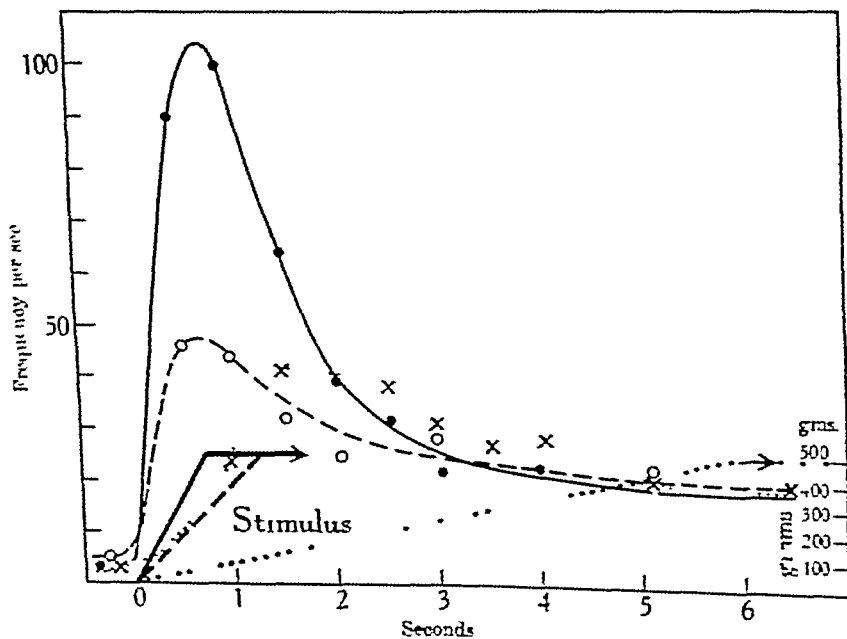


Fig. 8. Exp. 6. Frequency with different rates of increase of pressure. Every impulse counted.

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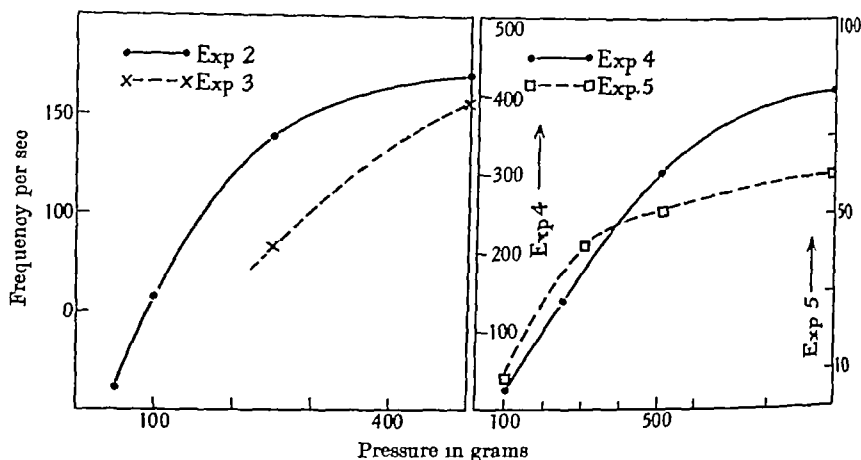


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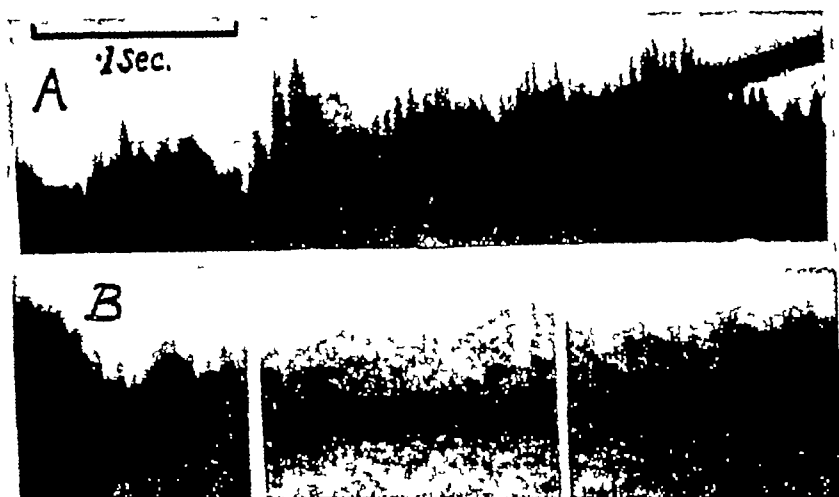


Fig 7 Film records of response to increasing pressure

A Exp 4. Rapid loading (500 grm in $\frac{1}{2}$ sec.) Interference of impulses as loading begins. Frequency has dropped before maximum pressure is reached. Black line signals increase of pressure.

B Exp 1. Slower rise of pressure (500 grm in 5 sec.) Loading begins in first section, midway in second, maximal in third. Frequency greatest at the beginning.

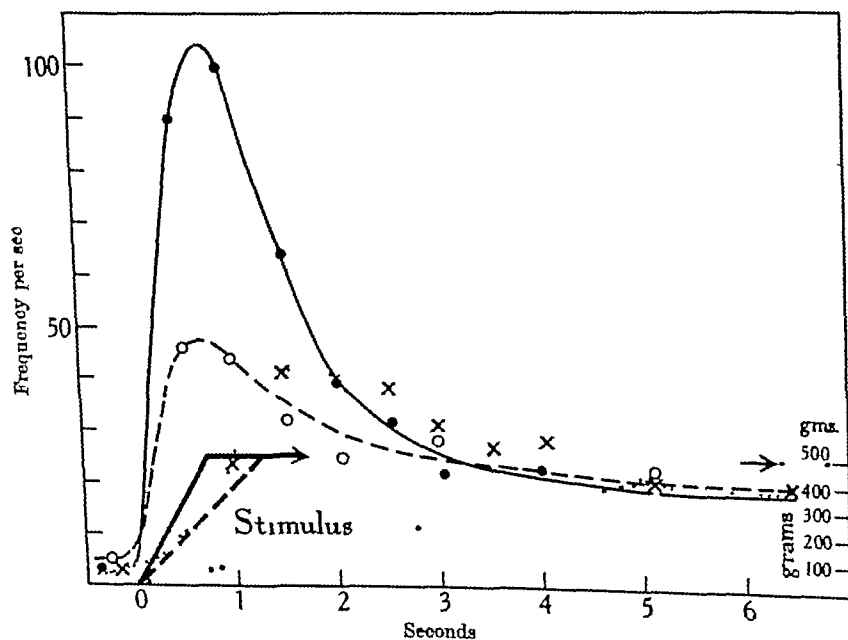


Fig 8 Exp 6 Frequency with different rates of increase of pressure
Every impulse counted.

has fallen considerably before the full loading is attained. It follows that the maximum frequency will depend much more on the rate of loading than on its final value. The curves in Fig 8 show an experiment in which the effect of altering the rate of loading was well marked. Fig 9 shows the effect of an increase in the final pressure in a preparation in which the adaptation was not so rapid. In two other experiments, however, although the maximum frequency was reached before the maximum

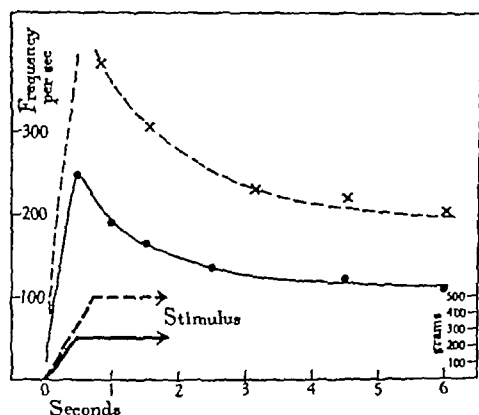


Fig 9

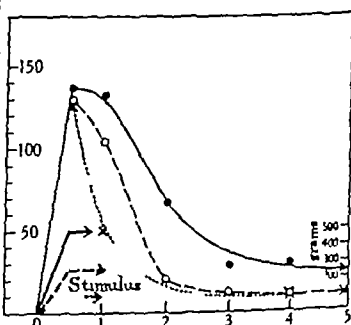


Fig 10

Fig 9 *Exp 4* Frequency with different final pressures. Top of upper curve obscured by signal

Fig 10 *Exp 1* Frequency reaches same (maximal?) value for all stimuli

pressure, it showed very little variation with changes in the rate of loading or in the final pressure. Curves from these experiments are given in Fig 10 (*Exp 1*). As soon as the pressure begins to increase, the frequency rises to a maximum which is much the same in every case. It then falls off rapidly if the final pressure is small, more slowly if the pressure is large. It is possible that our figures for the maximum frequency are at fault and that it is really higher with the more rapid loading. The impulses are certainly difficult to count and there may be more overlapping of impulses in some of the records. We are inclined to think, however, that the true explanation is that the stimulus was maximal in every case. The end organs cannot discharge impulses at more than a certain rate and this rate was already reached with the slow increase of pressure. This conclusion will be discussed in the section dealing with the frequency of discharge from individual end organs.

Since the rate of adaptation is rapid we might expect to find that the organs would return rapidly to the unadapted condition as soon as the

pressure is removed, and we should expect this too from a knowledge of the sensations we experience when the pad of the toe is pressed. If the pressure is removed and applied again immediately, the sensation rises again to somewhere near its initial intensity. In several experiments we have recorded the impulses from the cat's pad when the pressure is released and renewed after a short interval and there is no doubt that the state of adaptation does pass off very rapidly. An experiment of this kind is shown in Fig 11 (Exp 7) and it will be seen that when the pressure is renewed after an interval of 2 seconds' rest, the frequency rises very nearly to its initial maximum. Fig 12 (Exp 4)

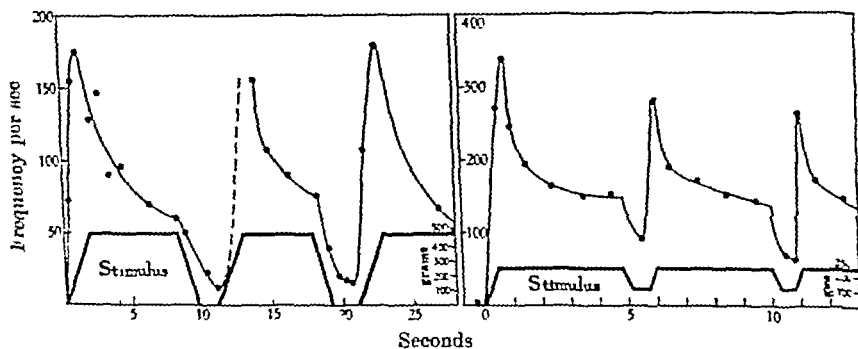


Fig 11

Fig 12

Fig 11 Exp 7 Complete removal of stimulus for short periods. Frequency returns to initial value when stimulus is renewed.

Fig 12. Exp 4. Partial removal of stimulus. Adaptation persists, but frequency rises again when full load is applied.

shows the effect of a partial removal of the pressure for a short period. Here the state of adaptation persists to some extent since the load is never removed entirely, but each rise of pressure brings about a large increase in frequency. This experiment is interesting because it enables us to form some idea of the nature of the adaptive process. This was discussed in Part II and it was pointed out that it might depend on a change in the rate of recovery of the end organs or on a change in the excitatory disturbance. We might suppose that the intensity of the excitatory disturbance is always proportional to the pressure at any moment, but that the refractory period increases very rapidly with activity and brings about the decline in frequency. The more likely explanation is that with a constant stimulus the excitatory disturbance diminishes rapidly so that the frequency declines, although the rate of recovery of the organs remains unaltered. This explanation is supported

by the record in Fig 12 Here the frequency has fallen to half its initial value in 5 seconds and if the exciting value of the stimulus remains unchanged, the refractory period of the end organs must have been doubled The drop in the stimulus from 250 to 125 grm could scarcely restore the end organs to near their resting condition, for the fall in frequency would still have taken place if the stimulus had been 125 grm throughout The frequency would rise when the stimulus is increased again to 250 grm but it would only rise to the value it had just before the load was reduced whereas in fact it rises a great deal higher The same difficulty does not occur if we suppose that the adaptation is due to the stimulus becoming less effective¹, for the end organs would then be capable of responding at any time with their original frequency provided that they received a sufficiently intense stimulus The fall in frequency with a constant pressure stimulus must therefore be due in part at least to a decline in the exciting value of the stimulus apart from any change in the refractory period of the end organs This agrees with the results obtained from the receptors in the frog's muscle and shows, as they did, that the adaptation of an end organ to a constant stimulus is of much the same nature as the adaptation of a nerve fibre to a constant current

Frequency of discharge from individual end organs Although the nerve under investigation has 200-400 fibres, it will be obvious from the data already given that very few of these fibres can be in action when the pad is stimulated by pressure The total number of impulses passing up the nerve in a second was often less than 50, and even if the frequency of the end organ discharge were as low as 5 per sec, this would mean that only ten organs were in action However, there is really no reason to credit the cat's toe with a large number of pressure receptors Owing to its power of responding with different frequencies, one end organ should be enough to signal the intensity of the stimulus More would be needed if the exact site of stimulation were to be signalled as well as the intensity, but it is not likely that there is a very accurate localisation of pressure within the small area supplied by the nerve (i.e. half the plantar surface of one toe)

Of the different types of sensory end organ in the subcutaneous tissue the Pacinian corpuscles are by far the largest, and we have tried several times to dissect out a single corpuscle in the living preparation and record the impulses from it, but in every case we have failed Damage to the

¹ The decline in effectiveness of the stimulus may be caused in part by the gradual yielding of the tissues for this might reduce the extent to which the pressure organs are deformed.

nerve and the end organ is a sufficient explanation of this, though it is conceivable that we were not using the right form of stimulus for this end organ

The failure to obtain records from preparations with only one end organ need not concern us so much, for those from the intact pad give most of the evidence we need. In the nerve from the frog's muscle (cf. Part II) the afferent impulses fell into several regular series each with a definite rhythm of its own, each series being produced by a single end organ. If the mammalian pressure receptor is built on the same plan we might expect to find evidence of regular rhythms in the present records and these would tell us what was happening in each end organ.

It must be said at once that the discharges cannot always be grouped into perfectly regular sequences. In one record (Exp 6) we have found a series of four consecutive impulses occurring with perfect regularity at intervals of 0.195 sec. during an increase of pressure of 100 grm. per

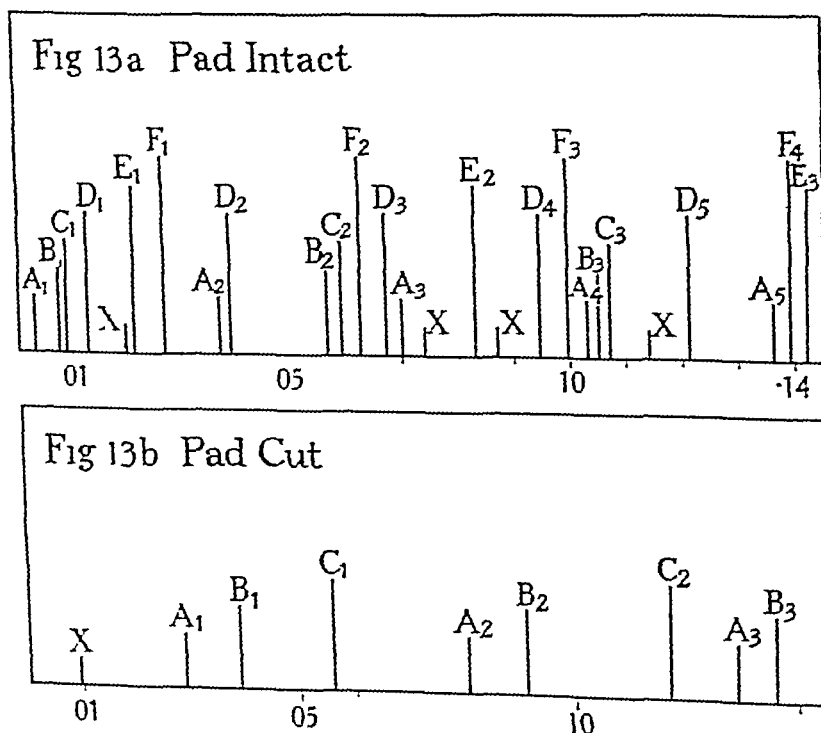


FIG. 13. Exp. 2. (a) Pressure of 500 grm. for 5 sec. Analysis of impulses into regular rhythms. (b) Distal part of pad cut through. Same stimulus.

sec In other records we can usually detect one or more distinct rhythms with intervals not varying by more than 5 p c , but most of the impulses will not fit into groups unless we allow a variation of rhythm of at least 10 p c during the short space of one record. An example will make this clearer. Fig 13 gives the moment of occurrence of all the impulses in a particular record. The stimulus was a pressure of 500 gm applied for 5 seconds. The impulses have been analysed into six groups marked *A-F* and the intervals between the successive impulses in each group are given below in Table I. The four impulses marked *X* which are left out of the scheme may belong to sequences of very slow rhythm. It will be seen that there is a variation of 10 p c in the intervals in series *F* and this is well outside the range of experimental error. The impulses may be wrongly grouped and it is conceivable that if 27 impulses had occurred at random during the period of the record, we should have been able to analyse them into groups having the same degree of regularity as those in Table I. But the experiments on the frog and the one record already mentioned (Exp 6) show that regular discharges are to be expected, and the periods found in Table I agree very well with those from other records.

At a later stage in the same experiment a deep incision was made

TABLE I

Exp 2 500 gm pressure applied for 5 seconds

a Pad intact.

Intervals between impulses in each group

sec	sec	sec	sec	sec
$A_1-A_2 = 034$	$B_1-B_2 = 049$	$C_1-C_2 = 050$	$D_1-D_2 = 026$	$E_1-E_2 = 061$
$A_2-A_3 = 033$	$B_2-B_3 = 0485$	$C_2-C_3 = 048$	$D_2-D_3 = 028$	$E_2-E_3 = 059$
$A_3-A_4 = 033$			$D_3-D_4 = 0275$	
$A_4-A_5 = 033$			$D_4-D_5 = 026$	
	sec			
	$F_1-F_2 = 0355$			
	$F_2-F_3 = 037$			
	$F_3-F_4 = 039$			
Average frequency				
<i>A</i> 30 per sec				
<i>B</i> 20.5 ,				
<i>C</i> 20 ,				
<i>D</i> 37 ,				
<i>E</i> 17 ,				
<i>F</i> 27 ,				

b Pad cut across Same stimulus

Intervals between impulses in each group

sec	sec	sec
$A_1-A_2 = 0515$	$B_1-B_2 = 052$	$C_1-C_2 = 061$
$A_2-A_3 = 0485$	$B_2-B_3 = 045$	
Average frequency		
<i>A</i> 20 per sec.		
<i>B</i> 21 ,		
<i>C</i> 16.5 ,		

across the pad, cutting away the distal two-thirds from connection with the nerve trunk. The responses to the same stimulus are given in Fig 13 (b). They now fall into three instead of six groups, and the periods are given in Table 1 b, but there is again a distinct variation in the intervals and a corresponding doubt as to the correct grouping. In other records the variation may be even greater and it becomes quite impossible to group the impulses with any confidence.

In Table II we have collected various regular frequencies found in different experiments. As a criterion of "regular" frequency we take the occurrence of at least four impulses in the record at intervals which vary by less than 10 p.c. When only one frequency is given for a particular record it means that only one could be found to satisfy this criterion. The lower frequencies do not appear in the table, for the rate must be higher than 20 per sec. if four impulses are to appear on the photographic plate.

TABLE II.

Number of experiment	1	5	6	2	7	8
Stimulus	Frequency per second					
Slow increase of pressure 100 grm. per sec.	56	61	52	—	62.5	54
500 grm. constant load for $3\frac{1}{2}$ sec.	—	36	—	—	—	—
Do. for 5 sec.	—	—	—	37 30	—	—
Do. for 7 sec.	—	21	—	—	—	—
Do. for 10 sec.	—	—	—	—	—	26.5 26

The frequencies obtained whilst the pressure was increasing are in remarkably close agreement, and it is unlikely that any higher frequencies can have been overlooked, for in many of the records there are occasional quiet periods lasting as long as 0.1–0.15 sec. The exact pressure at the moment of exposure was not recorded on the plates and may have varied from 150 to 350 grm., but the curve in Fig 9 shows that the frequency does not vary much when the pressure is increasing slowly between these values. A more rapid increase of pressure would no doubt give higher rhythms, but before the maximal rate of the end organ is discussed we have to consider why the rhythms we have found are on the whole less regular than they were in the frog.

Irregular responses. In the records from the frog some irregularity was present when the frequency was very low, and whatever was the cause of this may account for the present records also. But there are other possibilities which must be considered. It is unlikely that the more rapid adaptation can account for the lack of regularity, for the total

frequency does not fall by more than 2 or 3 p.c. in a period of 16 sec (the duration of the record). If the rhythms were all very long, we should not be able to trace them in records lasting only 16 sec, but we have looked for slow rhythms in the continuous film records and have not found them. A more probable explanation is supplied by the fact that we are dealing with nerve fibres each of which may send branches to several end organs. This arrangement is not found in the case of the muscle spindle and indeed Sherrington⁽³⁾ has shown that in the cat the converse is often true, one muscle spindle receiving several afferent nerve fibres. Thus, in our records from the frog the possibility of interaction between different end organs did not arise. It must arise, however, when we are concerned with the end organs under the skin, for it is well known that both the Golgi-Mazzoni and the Pacinian corpuscles may occur in groups supplied by a single nerve fibre, and an impulse arising in one corpuscle could scarcely fail to modify the activity of the others¹. This explanation is supported by the data obtained for the maximal frequency of the response.

Maximal frequency of end organ response. In most of our experiments a rapid rise of pressure (100 grm. in 1 sec.) gave a total frequency of more than twice the rate given by a slower rise (100 grm. in 1 sec.). Part of this increase in frequency might be due to new organs responding only to a rapidly changing stimulus, but in any case the maximal rate at which the single organ can discharge ought to lie somewhere between 60 and 150 per sec. But in Exp. 1 (Fig. 10) and Exp. 6 (Fig. 9) it will be seen that the total frequency with rapid loading does not exceed 150 per sec. It is unlikely that the stimulus affected only one pressure receptor and the impulses did not form a single regular series, but here, too, we may invoke the fact that several end organs may have a common nerve fibre. An impulse set up in one organ will almost certainly be conducted round as an antidromic impulse to the other organs on the same fibre. If it reaches an organ which is in the refractory state, the antidromic impulse would have no effect, but if the absolute refractory period is over, the impulse might produce a renewed activity of the organ and a renewed refractory period. It does not follow that a fresh centripetal impulse would be set up, for the refractory state of the conducting path would prevent it. Thus the total frequency of the impulses travelling up the nerve fibre from the group of organs might be no greater than the maximal frequency at which any one organ can respond. Since the

¹ The possibility of interaction of end organs on a common nerve fibre was pointed out by Herring (Brain, 46 p. 209, 1923).

impulses occur irregularly, it is unlikely that one organ can keep all the others permanently out of action, but it is interesting to note that Exp 6 (one of those with a very low total frequency) gave the record in which four consecutive impulses appeared at perfectly regular intervals. The film records show that this regularity was not maintained, and we must therefore assume that the lead passes from one organ to another. For this to occur we must also assume that the refractory state set up by an antidromic impulse is sometimes shorter than that set up in the organ which originates the impulse.

Since the whole explanation is speculative, it would be useless to discuss in detail the way in which several end organs might interact. It may be pointed out, however, that a disordered rhythm in a nerve fibre attached to several end organs presents a fairly close analogy with the behaviour of the ventricle of a heart in which the rhythm is disturbed by ectopic beats from various parts of the auricle.

Whether this explanation is correct or not, the fact remains that in these two experiments the total number of impulses passing up the nerve in 1 sec. was not greater than 150, and the maximal rate of discharge from the single organ cannot well be higher. There was nothing to indicate that the preparations were in any way abnormal and the regular periods obtained in other experiments are certainly no greater. A maximal frequency of 150 per sec. for a warm-blooded animal is evidently in good agreement with that for the receptors of the frog's muscle, which was reckoned to be 75-100 per sec.

Discussion. The results obtained from the pressure receptors of the cat's foot conform very closely with those for the tension receptor in the frog's muscle. The rate of adaptation is more rapid and the maximal frequency of discharge is probably higher, but there is the same grading of frequency according to the strength of the stimulus and the same all-or-nothing relation between the stimulus and the individual responses in the nerve fibre. We see no reason to doubt that the mode of production of the repeated discharge is the same in both, i.e. after each response the refractory state is set up and the excitability returns gradually until it reaches the value at which the stimulus becomes effective again. In both cases the change which would be produced in the end organ by the stimulus is a deformation of its structure. The muscle spindle will elongate when the tension on the muscle is increased, and it will do so whether the muscle is contracting or not, since it is placed as a link between two sections of a contractile fibre. The pressure organ must also be deformed if the stimulus is to be effective, since it

is well known that an increase of pressure does not stimulate if it is uniform in all directions, *e g* if the limb is placed in water or mercury. The exact process by which the deformation sets up an impulse is, of course, uncertain.

The main difference between the frog records and those from the cat lies in the absence of clear evidence of regular rhythms except in a few cases. This has already been discussed and it has been pointed out that several pressure organs may interfere with one another if they are supplied by a common fibre. In our records from the frog we found that the frequency of discharge from the muscle spindle was always low enough to leave the nerve fibre time for complete recovery between one impulse and the next. This condition would evidently not obtain if a single nerve fibre were to receive impulses from several end organs acting quite independently, but it would be secured if the nerve endings on a common fibre can interact in the way suggested.

If it is true that the activity of one end organ may suppress that of the others on the same nerve fibre, it follows that the multiplication of end organs on a single fibre would merely serve to increase the area upon which the stimulus might take effect. The frequency of discharge would be the same whether all were stimulated or one only, and presumably the intensity of the sensation and its "local sign" would be the same also.

The very rapid adaptation to contact and pressure is surprising at first sight, but it becomes less so when we consider the functional value of these sensations in the cat's toe. In our own toes the sensation due to a steady pressure is very soon over unless it is renewed by a movement, and although we have no objective standard it is fairly clear that the sensation does vary with the intensity and duration of the stimulus according to the same general plan which governs the frequency of discharge of impulses in these experiments.

CONCLUSIONS

1 Afferent impulses have been recorded in the plantar digital nerves of the cat when the pad of the toe is stimulated by light contact or by pressure.

2 When a glass disc is brought into contact with the pad and rests there lightly, there is a discharge of impulses at a high frequency for a period of about $1/10$ – $1/5$ sec at the moment of contact, but within $1/2$ sec the discharge has ceased almost entirely.

3 When pressure is applied to the disc, a discharge of impulses takes

place which is at a maximum as the pressure is increasing and falls off rapidly when the pressure is maintained at a constant value

4 The frequency of the impulses varies with the intensity of the stimulus, but the size of the individual action currents does not vary. There is therefore an all-or-none relation between the stimulus and the impulse

5 The discharges can sometimes be analysed into groups, each occurring with a definite rhythm and each presumably due to a single end organ, but the rhythms are not so regular as in the afferent fibres from the frog's muscle and it is often impossible to trace them. It is suggested that this lack of regularity is due to interaction between several end organs which are supplied by a common nerve fibre

6 The maximal rate of discharge from the single end organ appears to be about 150 per sec. Frequencies of 20-30 per sec are commonly found when the pressure has been constant for 5 seconds

7 These results agree very closely with those obtained from the tension receptors in the frog's muscle and there is little doubt that the activity of the cat's pressure receptors depends in the same way on the development of a refractory state and a return of excitability after each discharge, combined with a slow decline in the exciting value of the stimulus

The expenses of this research were defrayed in part by a grant to one of us (E. D. A.) from the Government Grants Committee of the Royal Society

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THE INFLUENCE OF CALCIUM ON THE ISOMETRIC RESPONSE OF THE FROG'S HEART

By D E DESEÖ (*Fellow of the International Educational Board*)

(*From the Pharmacological Department, University College, London*)

THE following experiments were made to determine the effects of variations in the calcium content of Ringer's fluid upon the isolated ventricle of *Rana temporaria*

The whole ventricle was perfused with a Kronecker's cannula, and the isometric responses were recorded with a Frank's manometer. The arrangement used was similar to that described by Frank(1), and modified by Kozawa(2) and by Segall(3). A constant rhythm was maintained by stimulation with break induction shocks at 12 per minute. The excursions of the lever were recorded on a fast drum, and the records were measured by means of a reading microscope and micrometer.

The Ringer's fluid was buffered with borates and acetates and had the following percentage composition: NaCl 0.65, CaCl_2 0.014, KCl 0.014, H_3BO_3 0.031, $\text{C}_2\text{H}_3\text{O}_2\text{Na}$ 0.068, p_{H} 7.6

The isometric response of the heart with a series of different fillings was measured, and the effect of alterations in the calcium content was determined by making series of experiments alternately with different calcium concentrations.

Three concentrations of calcium chloride were tested, namely, (1) *normal* 0.014 p.c., (2) *excess* 0.042 p.c. and (3) *deficiency* 0.004 p.c.

Doi(4) pointed out that a series of isometric responses produced considerable fatigue of the ventricle, particularly when maximal fillings were used. I noted this effect also, and usually made several series of experiments alternately with two solutions. The results shown in the figures were selected from experiments in which changes due to fatigue in the course of a single experiment were not marked. Excess of calcium in a fresh heart beating vigorously caused an increase in diastolic pressure but did not cause any marked increase in systolic pressure (Figs 1 and 3). Excess of calcium in a fresh heart beating feebly (Fig 2), or in a fatigued heart (Fig 4), caused a rise in diastolic pressure and also a considerable increase in systolic pressure.

Excess of calcium was found to produce less effect in a neutral

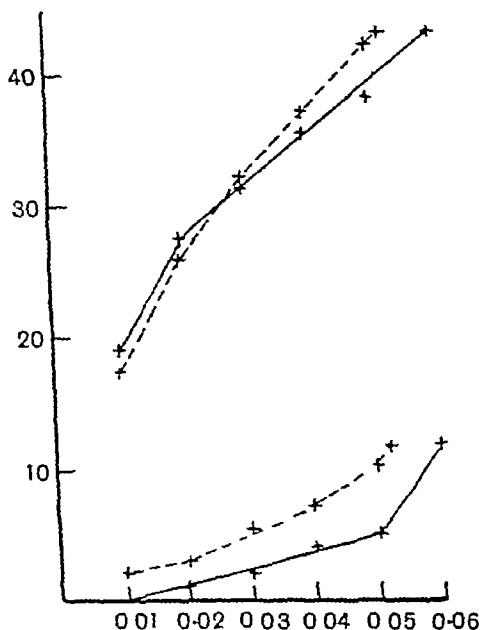


Fig 1 Effect of excess of calcium on fresh heart. First series (continuous lines) $\text{CaCl}_2 = 0.014$ p.c. Second series (broken lines) $\text{CaCl}_2 = 0.042$ p.c. The upper lines show systolic pressures and the lower lines diastolic pressures Ordinate pressures in mm Hg Abscissa filling of ventricle in c.cm.

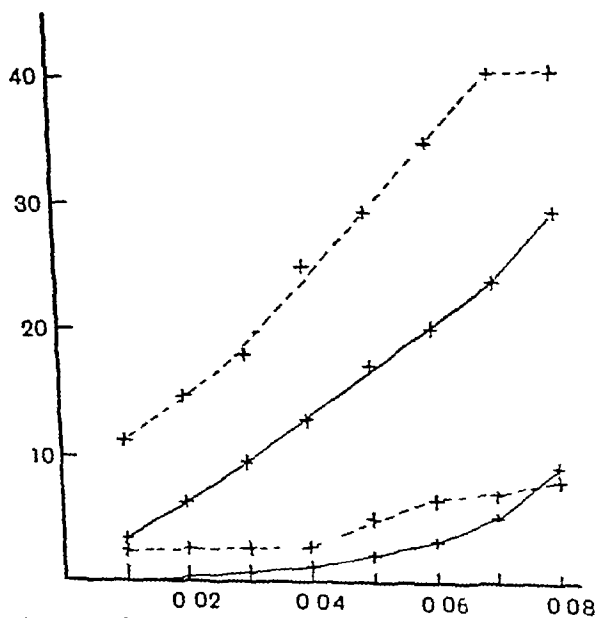


Fig 2. Heart giving feeble contraction with normal Ringer. Curves and coordinates as in Fig 1. First series (continuous lines) $\text{CaCl}_2 = 0.014$ p.c. Second series (broken lines) $\text{CaCl}_2 = 0.042$ p.c.

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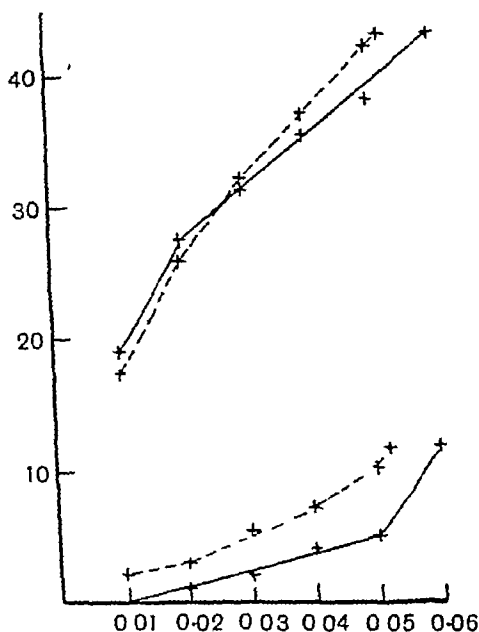


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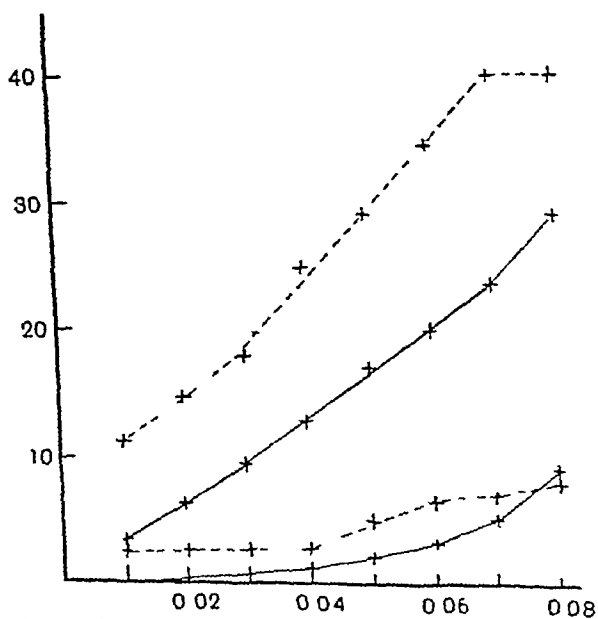


Fig 2 Heart giving feeble contraction with normal Ringer. Curves and coordinates as in Fig 1. First series (continuous lines) $\text{CaCl}_2 = 0.014$ p.c. Second series (broken lines) $\text{CaCl}_2 = 0.042$ p.c.

solution (p_H 7.0) than in an alkaline solution (p_H 7.8). Deficiency of calcium produced no certain effect on the diastolic pressure, but caused

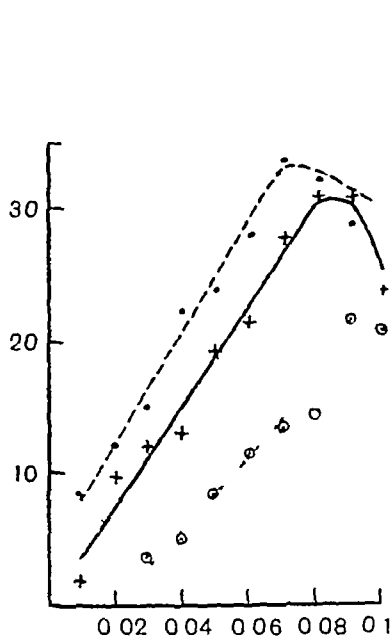


Fig 3

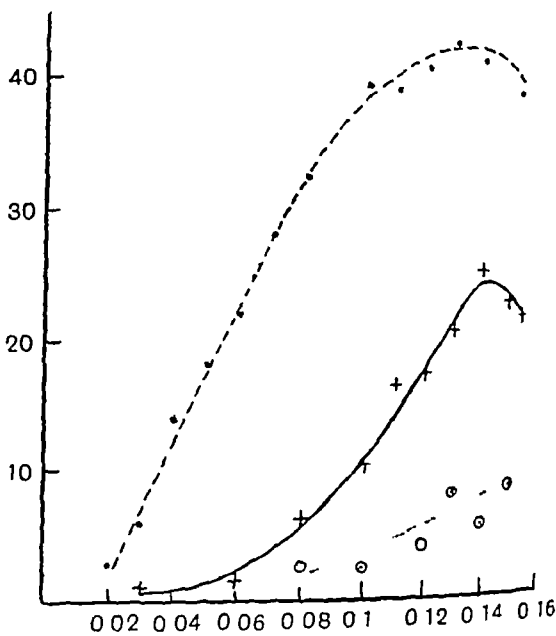


Fig 4

Fig 3 Pressures added on contraction in fresh heart. Coordinates as in Fig 1
 ----- $\text{CaCl}_2 = 0.042$ p.c., + — + $\text{CaCl}_2 = 0.014$ p.c., \odot \circ $\text{CaCl}_2 = 0.004$ p.c.

Fig 4 Pressures added on contraction in fatigued heart. Curves and coordinates as in Fig 3

a great decrease in the systolic pressures (Figs 3 and 4). Kozawa⁽²⁾ showed that increase in the filling of the frog's heart up to a certain optimum caused an increase in the systolic pressure produced. Figs 1 and 2 show that calcium excess diminishes slightly the quantity which is an optimal filling for the heart.

Excess of calcium, as is well known, increases the duration of the isotonic contraction of the frog's ventricle and it produces a similar effect on the isometric response. Increased filling of the heart increases the duration of the isometric response and Segall⁽³⁾ has shown that this increase is due chiefly to an increase in the duration of fall of tension. This effect is exactly similar to that seen in skeletal muscle. Fig 5 shows that excess of calcium increases and calcium deficiency decreases the duration of the isometric response, and that these changes are due chiefly to changes in the duration of the fall of tension.

CONCLUSIONS

(1) Calcium excess causes a slight diminution in the resting length of the heart muscle, as is shown by the fact that the diastolic pressure for any particular filling is increased

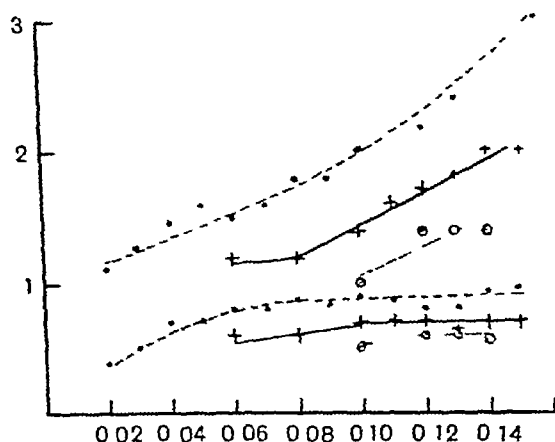


Fig. 5 Influence of calcium concentration on the duration of contraction (same heart as in Fig. 4)

--- $\text{CaCl}_2 = 0.042$ p.c., + — + $\text{CaCl}_2 = 0.014$ p.c., ○ — ○ $\text{CaCl}_2 = 0.004$ p.c.
Ordinate time in seconds, abscissa filling of ventricle in c.cm. The figure shows three different pairs of curves. The upper curves show total duration of rise and fall of tension and the lower curves the duration of the rise of tension.

(2) The effect produced by calcium excess upon systolic pressure depends upon the condition of the heart. Little increase is produced in a fresh heart beating vigorously but a considerable increase is produced in a heart beating feebly and particularly in a fatigued heart.

(3) Calcium excess reduces the quantity of filling which produces an optimal systolic response.

(4) Calcium deficiency reduces the systolic pressures produced by the heart.

(5) Calcium excess increases and calcium deficiency decreases the duration of the isometric response, and this increase is due chiefly to an increase in the duration of the fall of tension.

I desire to thank Prof. A. J. Clark and Dr G. Anrep for their valuable help and advice, and Dr Segall for communicating to me certain unpublished conclusions.

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SECRETIN AND THE PORTAL CIRCULATION

By J MELLANBY

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IN 1917 Djenab⁽¹⁾ stated that secretin injected into the portal circulation is a much less effective stimulant of pancreatic secretion than when injected into the jugular vein. Since the obvious path for the absorption of secretin from the intestine is *via* the portal blood, his results indicated that secretin is not necessary for the production of pancreatic juice in the normal animal. In 1921 Halliburton and de Souza⁽²⁾ observed that secretin injected into the splenic vein produced less pancreatic juice than when injected into the jugular vein. In their opinion the extra dilution of the secretin injected into the portal blood before its arrival at the pancreas would explain their results. In 1924 Cowgill and Deuel⁽³⁾ found a substantial diminution in the amount of pancreatic juice formed when secretin was injected into the splenic vein, compared with that produced when the same quantity of secretin was injected into the jugular vein. In one experiment on a dog, 10 c c of secretin injected into the femoral vein produced 41 drops of pancreatic juice, whilst the same quantity of secretin injected into the splenic vein produced 7 drops of pancreatic juice. They suggest that the portal path of absorption should receive due attention from investigators engaged in the study of secretin.

In order to clear up the ambiguity on this subject, experiments were carried out to determine the relative parts played by the liver, the portal circulation and the lymphatic system in the secretin mechanism for the production of pancreatic juice.

Experimental methods The experiments were carried out on cats anaesthetised by urethane (1.5 grm per kilo of body weight). The rate of secretion of pancreatic juice was determined by timing the drops issuing from a cannula tied into the pancreatic duct. Injections of secretin were made into a cannula tied into the right femoral vein or directly into a small branch of the mesenteric vein through a fine needle. The crude secretin solution was made by extracting the duodenal mucous membrane of a pig at room temperature with absolute alcohol.

Secretin into the femoral vein and mesenteric vein The following results

show the marked difference in the amounts of pancreatic juice secreted when secretin (2 c c) was injected into the femoral vein (A) and the mesenteric vein (B)

Pancreatic juice in drops	(A)		(B)	
	m.	s	m	s
1	1	27	2	6
5	2	42	5	27
10	3	47	12	7
15	5	48	—	—
20	8	5	—	—
25	10	17	—	—
30	12	58	—	—
35	17	30	—	—

Figures similar to the above were obtained in a series of experiments. They fully confirm the results of previous observers. Two deductions may be made from these experimental results: (1) that the liver has first claim on secretin and, only when this claim is satisfied, the excess of the secretin in the portal blood passes through the liver to the pancreas, or (2) that in the normal animal secretin is absorbed from the intestine into the lacteals, and passes indirectly into the blood through the thoracic duct.

The liver and secretin. According to Bayliss and Starling⁽⁴⁾ secretin acts on the pancreas and liver since an intravenous injection of it not only causes a secretion of pancreatic juice but also doubles the rate of flow of bile. It appeared possible, therefore, that a single injection of secretin into the portal circulation would stimulate the secretion of bile rather than that of pancreatic juice. This hypothesis was tested by determining the relative rates of secretion of pancreatic juice and bile after the injection of secretin (1 c c) into the femoral vein (A) and into the mesenteric vein (B).

Pancreatic juice in drops	(A)		(B)	Bile in drops	(A)		(B)
	m	s			m	s	
1	1	2	1 55	1	3	10	12 0
5	2	12	5 55	2	7	5	—
10	4	14	20 30	3	11	45	—
15	6	36	—	4	16	9	—
20	9	52	—	5	30	25	—
25	15	33	—				
30	29	30	—				

It is evident that secretin injected into the mesenteric vein is a much less effective stimulant, not only for pancreatic juice but also for bile, than when injected into the femoral vein.

Secretin and the lymphatic system. The foregoing results indicated that secretin may be absorbed into the lymphatic system. This path of

absorption would explain the large secretion of pancreatic juice which follows after a meal of fat. It is impossible to inject secretin directly into the lacteals of the small intestine, but a method of testing this hypothesis was indicated by the experimental results and conclusions described in a previous paper (Mellanby(5)). It has been shown that bile of an appropriate reaction, injected into the duodenum, causes a copious flow of pancreatic juice. Experimental analysis of the phenomena indicates that bile salts are absorbed from the intestine and in their passage through the cells of the duodenal mucosa carry the pre-formed secretin contained in these cells into the portal blood or lymph and so to the pancreas. In the original experiments it was assumed that the absorption was into the portal blood. In view of the results described above, however, it was necessary to test the accuracy of this assumption.

The effect of clamping the thoracic duct Dilute bile (2 c c ox bile, 8 c c 85 p c NaCl, 05 c c HCl N) was injected into the duodenum of a cat. After the secretion of 2 c c of pancreatic juice, the left thoracic duct was clamped, and, after the secretion of an additional 2 c c of pancreatic juice, the clamp was removed.

Pancreatic juice in drops	Time in minutes	Thoracic duct
40	20	Free
40	15	Clamped
40	18	Free

The results indicate that secretin is absorbed directly into the portal blood, and that none passes indirectly into the blood through the lymphatic system of the intestine.

The effect of collecting the lymph from the thoracic duct It appeared possible that secretin passed into the portal blood when the free flow of lymph from the thoracic duct was prevented by a clamp. This possibility was tested by tying a cannula into the thoracic duct and allowing the lymph to flow freely from it during the secretion of pancreatic juice, produced after the injection of dilute bile into the duodenum.

Dilute bile, as in the previous experiment, was injected into the duodenum of a cat. When the pancreatic juice was flowing freely a cannula was tied into the thoracic duct and the lymph collected. The following figures show the effect of this procedure on the flow of pancreatic juice.

Pancreatic juice in drops	Time in minutes
16	24
16	27*

* Lymph flowing freely from thoracic duct

The lymph secreted during the experiment, injected directly into the blood, caused no secretion of pancreatic juice. It is evident that the removal of lymph from the thoracic duct has no effect on the secretion of pancreatic juice consequent on the injection of bile into the duodenum. Therefore, if secretin is the normal pancreatic stimulant, it must be absorbed directly into the portal blood and not into the lymphatics of the intestine.

Purified secretin injected into the mesenteric vein. The previous experiments offer conclusive proof that secretin is carried from the intestine to the pancreas by the portal blood. On the other hand, secretin extracts injected into the portal vein stimulate the pancreas to secrete only about one-third the quantity of juice obtained when a similar injection is made into the femoral vein. The explanation of these contradictory results is evident from the following figures, which show the relative rates of secretion of pancreatic juice after the injection of purified secretin (1 c.c.) into the femoral vein and the mesenteric vein.

Pancreatic juice in drops	Secretin into femoral vein		Secretin into mesenteric vein	
	m	s	m	s
5	2	30	3	0
10	5	12	5	0
20	7	57	9	3
30	10	58	13	25
40	14	20	18	37
50	16	50	26	56
60	26	20	—	

It is evident that purified secretin injected into the mesenteric vein is approximately as effective a stimulant for the pancreas as when injected into the femoral vein. Probably the anomalous results obtained when crude secretin extracts are injected into the two circulations are due to the association of the secretin, in such extracts, with other substances which are removed from the portal blood when passing through the liver, and hence the secretin also is removed from the circulation.

SUMMARY

(1) Secretin is absorbed from the cells of the mucous membrane of the small intestine directly into the portal blood, since interference with the lymphatic circulation does not prevent the secretion of pancreatic juice after the injection of bile into the duodenum.

(2) Purified secretin is approximately as effective a stimulant for the secretion of pancreatic juice when injected into the portal as into the femoral vein.

(3) Crude extracts of secretin act as feeble excitants of pancreatic secretion when injected into the portal circulation, since the liver removes from the blood substances with which the secretin is associated in these crude extracts

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A NOTE ON THE ELASTICITY OF SKELETAL MUSCLES BY A V HILL

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IN a recent paper by Lindhard and Möller⁽¹⁾ objection was raised to the experiments of Gasser and A V Hill⁽²⁾ on the ground that no account was taken in them of the alleged possibility "that stretching may cause a muscle to contract" In the experiments in question the muscle was already being subjected to a continuous super-maximal tetanic stimulus applied at both ends, and, under such conditions, Wyman⁽³⁾ has recently shown that a stretch considerably diminishes the heat production, so cannot possibly be regarded as further "stimulating" the muscle If the resting muscle were "stimulated" by the stretch the result would be to make its behaviour similar to that of the tetanised muscle, and not unlike it as we showed there is, moreover, no evidence that stretching a resting skeletal muscle excites it, as admittedly it may do in a smooth muscle

A very serious objection, however, does exist in regard to the interpretation placed by Lindhard and Moller on their own experiments It can be shown that their results do not, in fact, measure the elasticity of the muscle at all, but may be deduced directly from the dynamical properties of a bundle of flexible but unextended fibres, carrying a swinging system of the type employed by them, and oscillating under gravity

Consider a cylindrical bundle of parallel fibres of length l fixed at the top and carrying at the bottom a swinging device of moment of inertia MK^2 When this is twisted the fibres are caused to lie in spirals in the cylinder, instead of in lines parallel to its axis, and since they are under tension (to support the weight of the swinging device) each now exerts a horizontal component tending to resist the twist The restoring couple is proportional to the amount of twist, and the system carries out a harmonic oscillation of period depending upon the dimensions of the bundle of fibres and the value of g The elasticity of the fibres does not come into the equations at all, and the results of Lindhard and Möller may be explained, qualitatively and quantitatively, on

simple dynamical principles, without any reference to the elastic properties of the muscle

In a ring of fibres between the radii r and $r + \delta r$ the number of fibres may be taken as proportional to $2\pi r \delta r$ the total tension exerted by them (their share of the weight of the swinging device) will be proportional to their number, and equal to $2\pi T r \delta r$ where T is the load per sq cm of section of the bundle. When the lower end is twisted through an angle θ the couple exerted by the horizontal components of all the forces in the individual fibres of the ring will be $2\pi T r^3 \theta \delta r / l$, since each fibre now makes an angle $\cos^{-1} r \theta / l$ with the horizontal. Considering the whole bundle the total couple must be

$$\int_0^r \frac{2\pi T r^3 \theta dr}{l} = \frac{2\pi T r^4 \theta}{4l}$$

But $\pi r^2 T$ is equal to the weight of the swinging device, Mg , so the couple becomes $Mg r^2 \theta / 2l$. Moreover $\pi r^2 l$ is equal to the volume of the bundle, say V , so that the couple finally becomes $Mg V \theta / 2\pi l^2$

The equation of motion therefore is

$$MK^2 \frac{d^2 \theta}{dt^2} = \frac{Mg V \theta}{2\pi l^2},$$

which is that of a harmonic oscillation of half period

$$t = \pi \sqrt{\frac{2\pi l^2 K^2}{gV}},$$

the M 's cancelling out

Introducing the values of π and g this becomes

$$t = 0.252 l K / \sqrt{V}$$

In the experiments of Lindhard and Möller K had the value of 2.6 cm, so that finally

$$t = 0.65 l / \sqrt{V}$$

From this formula we see that t should be proportional to l . In Lindhard and Möller's experiments (Tables I-IV) the following values of t/l are calculated

I	1.03, 0.96, 1.05, 1.16, 1.23, 1.34, 1.07,
II	1.37, 1.54, 1.45, 1.51, 1.53, 1.35, 1.45,
III	2.36, 2.52, 3.13, 3.20, 2.99,
IV	2.24, 3.95, 3.18, 3.29, 2.58, 2.27

The constancy of the value of t/l in each experiment is quite as good as could be expected from the consistency of the observations themselves (see particularly Exp II). Thus the change in the time of oscillation when the muscle is stimulated is due simply to

the change of length of the muscle, and has nothing whatever to do with its elastic coefficient

That this is really the case is borne out by a further calculation. In the last formula above the volume of the muscle can be calculated from the values of t and l observed

$$V = 0.42/(t/l)^2$$

Taking the mean value of t/l in all the experiments (stimulated and unstimulated), viz 2.1, the value of V so calculated is 0.1 c.c., corresponding to a sartorius muscle of about 0.1 gm weight. Lindhard and Möller do not give the weights of the muscles they used, but 0.1 gm is exactly of the right order of size for the sartorius of an English frog, a coincidence which would be very strange were the above explanation not the true one.

SUMMARY

1 In a recent paper Lindhard and Möller have objected to the experiments of Gasser and Hill on the viscous-elastic properties of muscle, on the ground that "stretching may cause a muscle to contract". An answer is made to this objection.

2 Lindhard and Möller have proposed an alternative method of determining the elastic coefficient of muscle, at rest or stimulated. It is shown that in fact their results have no connection with the elastic properties of the tissue examined, but are due entirely to the dynamical properties of the system they employed.

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THE PRODUCTION OF SUGAR IN THE PERFUSED LIVER FROM NON-PROTEIN SOURCES

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It does not appear to be commonly known that when a liver is removed from the body and perfused with defibrinated blood at 37° , there is a production of sugar in the system greater than is accounted for by any disappearance of glycogen. This was first demonstrated by Embden(1) so long ago as 1905. He perfused the livers of dogs rendered glycogen-free before death by administering strychnine to the dogs after a period of starvation in which they were frequently exercised. At the beginning of perfusion he observed the absence of glycogen and determined the amount of free sugar in a lobe of the liver, and also determined the sugar in the blood which was used for perfusion. After perfusion for periods of $\frac{3}{4}$ to 1 hour he found an increase in the sugar in the blood much more than could be due to the breakdown of the traces of glycogen, or to the washing out of the free sugar initially present. In 1909, Lattes(2) extended these observations to the livers of phloridzinised and also of depancreatized dogs in which he encountered the same phenomenon. The results make it probable that the earlier findings of Seegen(3) in 1890 that there was an increase of sugar in pieces of liver tissue incubated at 37° from, e.g., an original value of 0.4 p.c. to 3.3 p.c., without any decrease in the amount of glycogen, were more accurate than some later observers have supposed.

Bornstein and Griesbach(4) have found in perfusing the livers of phloridzinised dogs that a fall in lactic acid takes place, and have supposed that the sugar production recorded by Embden was to be explained in this way.

Few other observers appear to have determined the total carbohydrate in the system of a liver perfusion both at the beginning and end. Bernhard(5), in an attempt to observe the effect of insulin on the rat's liver perfused with Ringer's solution, has done so, though he found that the increase of sugar in the perfusing fluid was accounted for by the diminution of glycogen.

Our experiments began with the purpose of finding out whether a perfused liver rich in fat would form sugar under the influence of such hormones as adrenaline or pituitary extract. It at once became clear that, to determine a change in the total carbohydrate in the system which could be regarded as significant, it was essential to reduce the initial total to the lowest dimensions. Thus in an experiment in which no precautions to ensure this were taken, we observed a rise in carbohydrate during the perfusion from 2.05 gm. to 2.49 gm., it was conceivable that the difference lay within the experimental error of the methods employed. Fortunately, we found that the livers of dogs or cats, fed for some days on fat, contained after removal from the animal little or no glycogen, it remained to reduce the total of free sugar in the liver before perfusion began. This object was achieved by perfusing once through the liver a portion of blood, which was discarded. The procedure proved to be of considerable importance because it simultaneously removed other diffusible constituents, such as urea, ammonium salts, amino acids and lactic acid, which might be present after a period of tissue asphyxia in abnormally large amounts.

We have been able by perfusion of livers prepared in this way to establish that a considerable formation of reducing sugar takes place, which in proper conditions is continuous and may be uniform during 3 or 4 hours. We have determined that the formation is in no way due to a simultaneous disappearance of lactic acid, and moreover that only a small fraction of the sugar can be derived from protein.

DETAILS OF EXPERIMENTS

Diet of animals. Cats were fed on thick cream, cod-liver oil and water for periods of 4-7 days. On this diet they developed a considerable acetonuria.

Dogs were fed on mutton fat and water for longer periods. There was a slight acetonuria in the first two or three days, but subsequently this disappeared. They ate the ration of fat with regularity. In contrast to this tolerance of a fat diet by adult dogs, we found that small puppies given cod-liver oil and mutton fat became comatose in 24 hours and died in 48 hours.

Preparation of liver. The cat or dog was anaesthetised with ether administered on an open mask, and cannulae were put into the external jugular vein and into either the carotid artery (dog) or the abdominal aorta (cat). As much blood as possible was collected from the artery in a basin and defibrinated. During the exsanguination, warm, sugar

free Ringer's solution was run into the vein, this enabled us to secure a larger volume of blood for perfusion. The amount added was in the proportion of about 75 c c of Ringer for a 3 kgm cat, from which we commonly obtained 150 c c of the blood-Ringer mixture. For the perfusion of the cat liver the blood of either one or two other cats was obtained in identical fashion. In the case of dogs, sufficient blood for the perfusion was always obtained from the one animal without the infusion of Ringer's solution. Nevertheless, the infusion was always carried out, in order to dilute the blood remaining in the liver.

The animal having been bled out in this manner, the abdomen was opened and the sheath of mesentery carrying the portal vessels was sought. From this, the common bile duct and the portal vein were dissected out and ligatured and divided as far from the liver as possible. The hepatic artery, and the inferior vena cava, both above and below the liver, were also ligatured and divided, but care was taken to leave the lymphatics free. The mesentery and diaphragm were now cut away, as close to the liver as possible, and the liver removed and weighed. A piece of glass tube was tied into the portion of thoracic vena cava to establish efficient drainage from the liver during the perfusion, as will be explained. A glass cannula filled with saline was tied into the common bile duct, and a second cannula filled with the defibrinated blood into the portal vein. A loose ligature was applied around the connections of the lobe or lobes of the liver which it was proposed to remove for analysis. In the case of the cat, we habitually chose the two small posterior lobes lying beside the right suprarenal, in the case of the dog we chose the large right lobe representing about one-third of the total liver weight. The liver was then transferred to the perfusion apparatus. The time taken in the manipulations described was usually from 20-30 minutes.

The perfusion apparatus This was a modification of the apparatus described by Burn and Dale(6) for perfusing the leg of a cat or dog. The blood from a reservoir, after being warmed to 37° by passage through a coil in a thermostat, passed through a bubble trap, from which samples of blood could be withdrawn and in which a thermometer was placed, and entered the portal vein of the liver. The liver was placed in a wax-coated zinc tray Z, shown in Fig 1. The tray was covered above by a glass lid L, and surrounded at the sides and below by the water in the thermostat T. The blood drained out of the liver by the glass tube G tied into the cardiac end of the inferior vena cava. This tube hung down inside the wider tube W which conducted the

blood to the oxygenating system placed below the level of the thermostat. In this way an efficient siphonage out of the liver was obtained. The oxygenating system was devised on the principle of that described by Hooker(7). Oxygen passed into it after bubbling through water at 37°C in order to saturate the gas with water vapour. In several experiments a mixture of oxygen with 5 p c CO_2 was used. The blood collecting in the bottom of the oxygenator was returned by a Dixon perfusion pump, working in conjunction with two glass valves, to the reservoir. A coil of silver wire coated with vaseline was placed at the entrance of the blood into the reservoir, as described by Burn and Dale, in this way frothing was avoided.

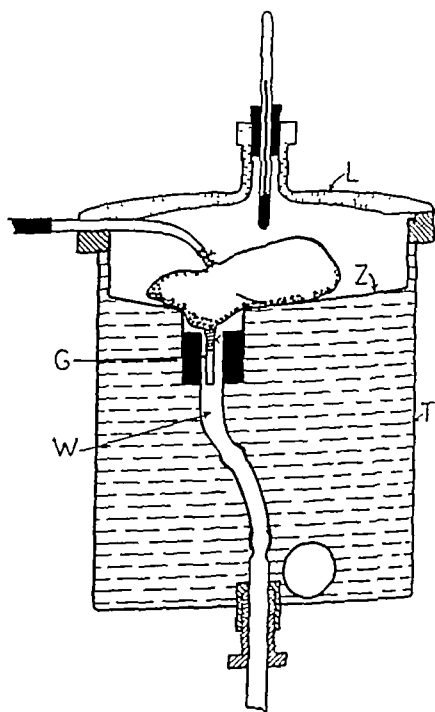


Fig 1 See text

The perfusion The usual rate of blood flow through the cat liver was about 120 c c per minute, through the dog liver the flow was somewhat greater than this, though not so much greater as we expected considering that the dog liver was about three or four times the size of the cat liver. About 200 c c of the blood-Ringer mixture obtained as described was used for each cat liver, and about 400 c c of practically undiluted blood for each dog liver. The cat livers showed much less tendency to become cedematous than did the dog livers. In those cases in which only very slightly diluted cat blood was used (which involved the exsanguination of two cats in addition to that from which the liver was taken) cedema, as determined by the initial and final weight of the liver, was negligible. A very successful perfusion of a dog liver in which there was little cedema was maintained for 4 hours by the addition of adrenaline to make a concentration of 1 in 3 millions. This addition was repeated at intervals of half an hour. Apart from this experiment there was usually considerable cedema in dog livers after 1 hour. Cedema

always occurred both in cat and dog livers if by accident the lymphatics running in the portal canal were obstructed by the ligature around the portal vein

The two best indices of efficient perfusion seemed to be the degree of the reduction of blood passing through the liver and the amount of bile flowing out of the cannula in the common bile-duct, between the two there was a general correspondence. That this flow was at least in part a true secretion and not a mere outflow from the gall bladder was evident from the facts, (1) that in one experiment there was a steady flow of bile from a second cannula placed in a tributary duct remote from the gall bladder, (2) that when the gall bladder was emptied by compression at the beginning of an experiment, as was often done, it filled again during the perfusion, (3) that the bile was light yellow in colour, unlike the green and viscid contents of the bladder. Grube⁽⁸⁾ has previously observed the filling up of the gall bladder with bile during a liver perfusion.

Methods (a) Determination of glycogen This was always estimated in portions of liver about 1 gm. in weight. Test-tubes were fitted with rubber stoppers having pieces of glass tubing about 3 ins. long passing through them to act as reflux condensers. In each test-tube was placed 1 c.c. of 60 p.c. KOH, the stoppers were fitted, and the tubes weighed. They were then placed in the water bath. When a sample of liver was cut out for estimation, it was put immediately into the tube. After 15 minutes in the water bath the tube was cooled and weighed again. The tube was then put back in the bath for 2-3 hours, and the estimation completed by Pflüger's method. The precipitation of glycogen was carried out in centrifuge tubes and the precipitate was collected and cleaned by centrifugation instead of filtration. Amounts so low as 0.05 p.c. glycogen were estimated in this way. Evans⁽⁹⁾ has recently pointed out that a very large error enters into the determination of small amounts of glycogen (at least so far as muscle glycogen is concerned), apparently due to the solubility of glycogen in 60 p.c. alcohol. Our glycogen estimations are therefore probably low, since, however, we habitually observed a higher figure for glycogen at the end of the perfusion, usually finding the liver free from glycogen at the beginning, it is also probable that the error had the effect of making the increase of total carbohydrate appear less than it actually was.

(b) Determination of free sugar in liver tissue This was estimated by placing a weighed piece of liver tissue, about 1 gm., in 5 c.c. of ice-cold 70 p.c. alcohol in a centrifuge tube of 15 c.c. capacity. The liver was cut

up into small pieces, and the fine scissors used for this were washed with a further 4 c c of the 70 p c alcohol, the washings being added to the tube. The small pieces were then crushed with a glass rod and the glass rod washed with a further $2\frac{1}{2}$ c c of alcohol, the washings again being added. The tube was corked and allowed to stand for 1 hour. At this stage the tube contained 10 grm liver and $11\frac{1}{2}$ c c alcohol. The tube was then spun in a centrifuge, being balanced against a tube containing water. Some alcohol was lost by evaporation during the spinning, and this was replaced by adding alcohol until the two tubes were again of equal weight. 10 c c of the alcoholic extract were removed with a pipette, and the alcohol was evaporated on a water bath. The viscous residue was taken up in 8 c c water and the protein precipitated with 2 c c of 0.5 p c dialysed iron. The precipitate was removed by filtration and the sugar in the filtrate estimated by the Shaffer-Hartmann method.

(c) *Determination of total carbohydrate* This was ordinarily estimated as the sum of the free sugar and the glycogen in the liver. The free sugar having been determined in the supernatant fluid as described, the residue in the centrifuge tube was used for determination of glycogen. The tube was placed in a water bath to remove the alcohol, 10 c c 60 p c KOH added, and the glycogen estimated as described above. Even in a liver from a dog fed on an ordinary diet the sum obtained in this way on any one sample is fairly representative of the whole liver, as the following example shows.

Lobe	Glycogen p c.	Free sugar p c.	Total p c.
A	3.796	1.169	4.965
B	4.724	0.539	5.263
C	4.062	0.604	4.666

In the livers of dogs fed on a fat diet, prepared as described, there was usually no glycogen, for the small amount present before death had undergone lysis during the period before perfusion began. Free sugar was present in corresponding amount, and in order to reduce this as far as possible, a portion of blood at room temperature was allowed to perfuse the liver once and was then discarded. The lobe for analysis was not removed until this had been done. The perfusion of the rest of the liver with oxygenated blood at 37°C then immediately began. The following figures show that the percentage of total carbohydrate in the lobe removed represents the percentage in other parts of the liver, for in these two experiments samples were taken from all four lobes of the liver after the excess of free sugar had been washed out.

	Lobe	Glycogen	Free sugar p.c.		Lobe	Glycogen	Free sugar p.c.
Exp 1	A	Nil	0.060	Exp 2	A	Nil	0.035
	B	"	0.068		B	"	0.029
	C	"	0.061		C	"	0.036
	D	"	0.053		D	"	0.030

There remained the possibility that carbohydrate existed in the liver other than glycogen and free sugar. In a liver from a dog fed on a normal diet we determined the carbohydrate present after prolonged hydrolysis of a portion of liver tissue in 5 p.c. HCl followed by defecation with sodium tungstate and compared it with the sum of glycogen and free sugar.

Glycogen p.c.	Free sugar p.c.	Sum p.c.	Total carbohydrate after hydrolysis p.c.
3.10	3.06	6.16	6.25
3.12	2.98	6.10	6.67
—	—	—	6.02

The sugar determinations in the solutions obtained after hydrolysis of the liver were apparently hindered by the presence of substances not removed by the Folin-Wu protein precipitation, the figures given are in each case the mean of several single determinations varying considerably from one another. We concluded that even in the case of a liver containing as much as 6 p.c. of carbohydrate, that portion not occurring as glycogen or glucose cannot be detected with certainty, and that in livers containing less than 1 p.c. it may therefore be neglected.

The increase in carbohydrate during perfusion. During the course of a perfusion lasting from 1 to 4 hours it was found that the total carbohydrate present in the system increased, rapidly at first and more slowly later. In experiments in which oedema occurred, after about 90 minutes the increase was entirely arrested, while in others where there was evidence of the persistence of a good functional condition the rise was maintained to the conclusion of the experiment. The following are two examples, these are described in detail to show in particular the method of arriving at the figure for the total carbohydrate. The initial total was always obtained in two ways. The final total was usually only determined in one way as in Exp 3, but in Exp 4 it was determined in two ways.

Exp 3. Dog 10 kgm. Fed on mutton fat only for 6 days.

Liver, initial weight before a lobe was removed, 274 grm. This was perfused once with 150 c.c. blood to wash out excess of free sugar, of this blood 56 c.c. remained behind in the liver, so that the total weight

of liver and blood was 330 grm. A lobe was now cut off for analysis, weight 98 grm, and perfusion begun with 370 c c blood in remainder. The actual liver perfused was taken to be 232 grm less the amount of 32 grm subsequently determined as being the weight of the gall bladder, portions of diaphragm, and connective tissue. The determination of total carbohydrate in the lobe removed gave the results

(a)	Glycogen nil,	free sugar	0 180 p c
(b)	"	"	0 180 "
(c)	"	"	0 178 "
(d)	"	"	0 180 "

The determination of sugar in the blood used for the perfusion gave the result 0 096 p c. Hence initial total of carbohydrate in the system was

(a)	In liver, 200 grm with 0 180 p c	= 360 mg
(b)	In blood, 370 c c with 0 096 p c	= 355 mg
Total		= 715 mg

The second estimation of this figure was obtained by examining the blood sugar after the perfusion had been in progress for 5 minutes. This interval allowed a sufficient time for the free sugar in the liver to be evenly mixed with the sugar in the blood. The sample was taken from the blood entering the liver and found to be 0 122 p c, calculating the total amount in 570 grm, this being the sum of weights of liver and blood, there was found to be 695 mg, a figure agreeing well with the previous estimate.

At the end of 1 hour's perfusion, during which the blood was powerfully reduced during passage through the liver and a flow of bile at the rate of four drops a minute was observed, the sugar in the circulating blood had risen to 0 245 p c, at the end of 2 hours 25 minutes the experiment was stopped, and the total carbohydrate again determined. The estimation of sugar in the liver being less accurate than in blood, after the perfusion was stopped and the blood removed from the apparatus, 200 c c of sugar-free Ringer were allowed to perfuse the liver and were then added to the blood. In this way the bulk of the liver sugar was transferred to the blood. The remaining liver sugar and the blood sugar were then determined. The last traces of sugar in the apparatus were calculated by circulating 100 c c of sugar-free Ringer for a few minutes, and estimating the sugar present afterwards.

Since the blood volume, including the 200 c c of Ringer, was 470 c c,

evidently 270 c c was the volume actually perfusing the liver before the perfusion ended. 34 c c had been removed during the experiment hence there were 66 c c of blood less than at the beginning. The liver weight, however, had increased by 55 gm., so there must have been a loss of 11 c c of water by evaporation in the oxygenating system.

The figures for the liver sugar in different lobes were (a) 0.145, (b) 0.079, (c) 0.049, (d) 0.066, the mean of these was 0.085 p c, giving on 255 gm liver, a total of 217 mg. The total carbohydrate at the end was found to be

In blood	1134 mg
In liver	217 ,
Removed during experiment	81 ,
Total	<hr/> 1432 ,

Hence after 2½ hours perfusion 717 mg appeared which were not present before, no allowance being made for loss of sugar by glycolysis or for usage of sugar by the liver's own metabolism.

Exp 4 Cat 3.0 kgm. Fed on cream for 4 days

Initial carbohydrate (a) The liver when prepared was found to be glycogen-free, and three samples in which sugar determinations were made gave the result (a) 0.153 p c, (b) 0.144 p c, (c) 0.121 p c or an average of 0.140 p c. The blood also contained 0.140 p c sugar.

Hence 0.140 p c sugar in a total of 56 gm. liver is 78 mg
and 0.140 p c sugar in a total of 200 c c blood is 280 ,

In all 358 ,

(b) After 5 minutes perfusion the blood contained 0.146 p c sugar, and this in 256 gm, the weight of liver and blood, represents a total of 373 mg.

Final carbohydrate (a) After 3 hours' perfusion the blood contained 0.288 p c sugar, 14 c c of blood were removed during the perfusion, hence the weight of blood and liver was 242 gm. The total of sugar was therefore, 697 mg, to which must be added 25 mg found in the 14 c c of blood removed. Hence the final total was 722 mg.

(b) By washing the sugar out of the liver with sugar-free Ringer as in Exp 3, and adding the washings to the blood, a total of 671 mg was found in the blood. Determination of the liver sugar gave 0.078 p c

as the average of the four figures 0.090 p.c., 0.091 p.c., 0.064 p.c. and 0.067 p.c. On 68 gm liver this was a total of 53 mg sugar. The blood removed during the perfusion and the last traces in the apparatus contained 52 mg sugar. Hence, by this procedure, a figure for the final carbohydrate total of $671 + 53 + 52 = 764$ mg was obtained.

Summarising, the initial carbohydrate was (a) 358 mg, (b) 373 mg, the final was (a) 722 mg, (b) 764 mg. The evidence is therefore strong that a rise of at least 349 mg in the carbohydrate content occurred.

We have observed this phenomenon in 22 experiments, in Table I appears a summary of the last eight which were performed. It will be seen that in all cases the two figures for the initial carbohydrate agree closely, and that the difference between them is very small when compared with the rise in carbohydrate observed.

TABLE I

Animal	Carbohydrate in mg				Period of perfusion hours	Hormone present
	Initial		Final	Gain		
	a	b				
Dog	533	559	1185	626	1.5	None
Cat	752	801	1246	445	2.5	,
Dog	1950	1944	3069	1119	1.5	"
Cat	877	858	1211	334	1.5	"
Dog	487	433	907	420	1.0	Insulin
"	1046	1014	2113	1067	1.5	Pituitary extract
Cat	798	767	1201	493	2.0	(Depancreatized)
Dog	—	997	2550	1553	3.0	Adrenaline and insulin

Influence of hormones It was surprising to find, as appears from Table I, that the production of sugar was not appreciably influenced by the presence of those hormones known to have an action in carbohydrate metabolism. One experiment, the beginning of which appears in Table I, was performed on the liver of a cat from which the pancreas had been removed three days before¹. The cat's own blood was used for perfusion fluid together with the blood of two normal cats which had had no food for the previous 24 hours. The rise of blood sugar was as follows:

Time	Blood sugar p.c.	Rise p.c.
5 mins after beginning	0.271	—
35 " "	0.364	0.093
65 " "	0.406	0.042
20 units of insulin added		
95 mins after beginning	0.432	0.026
125 " "	0.434	0.002

¹ This operation was kindly performed by Dr H. H. Dale.

The successive diminution of the half-hour rise in blood sugar which is recorded here, we observed in many experiments, and the figures give no evidence that the addition of insulin inhibited sugar production.

An experiment of greater value was performed on the liver of a dog, in which, after a large rise of blood sugar during the first half-hour, a uniform rise of blood sugar took place during five subsequent half-hour periods. During this time adrenaline and insulin were both added in order to discover whether it was possible to produce in the perfused organ the considerable accumulation of glycogen observed in the intact animal by McCormick, Noble and O'Brien(10). It was found that the addition of these hormones in no way affected the rise of blood sugar.

Time	Blood sugar p c.	Rise p.c. in 30 mins	Hormone
5 mins. after zero	0 176	—	—
35 " "	0 264	0 088	—
65 " "	0 302	0 038	—
			10 units insulin
			0.1 mg adrenaline
85 " "	—	—	0.2 mg adrenaline
95 " "	0 340	0 038	—
105 " "	—	—	0.2 mg adrenaline
125 " "	0 382	0 042	—
130 " "	—	—	10 units insulin
			0.2 mg adrenaline
185 " "	0 462	0 080	—
		(in 60 mins.)	

The rise in the reducing power of the blood In the experiments described we have spoken of a formation of sugar when the observation made has been of an increase in substances reducing copper. Holden(11) has shown that when glucose is estimated in this way in a fluid containing amino-acids the value obtained may be too high, the error amounting to 10–15 p c. He found that the method of Hagedorn and Jensen was unaffected by the presence of most amino-acids in moderate concentration. Since in a liver perfusion it was extremely likely that amino-acids were present, we compared the rise in the reducing power as estimated by the Shaffer-Hartmann method, which we ordinarily used, with that estimated by the Hagedorn-Jensen method.

First experiment

Time	Rise in reducing power expressed in mg per 100 c.c. blood	
	Shaffer Hartmann	Hagedorn-Jensen
1st half hour	54	56
2nd "	24	27
3rd "	21	16
	<hr/> 99	<hr/> 99

Second experiment

Time	Rise in reducing power expressed in mg per 100 c.c. blood	
	Shaffer Hartmann	Hagedorn-Jensen
3rd half hour	38	45
4th "	42	42
5th } "	80	83
6th }		
	<hr/> 160	<hr/> 170

The figures show that the reducing power estimated by Shaffer-Hartmann does not differ appreciably from that estimated by Hagedorn-Jensen, it is therefore probable that the reducing power determined is that due to sugar alone

Glycogen formation It is interesting to note that the formation of glycogen in a perfused liver was first observed by Luchsinger⁽¹²⁾, though that careful observer pointed out that his evidence depended on the assumption of an even distribution of glycogen throughout the different lobes. This assumption has since been shown to be justified by several workers, notably Grube⁽¹³⁾, Macleod and Pearce⁽¹⁴⁾, Grube⁽¹⁵⁾ and Barrenscheen⁽¹⁶⁾, working on the dog liver, observed glycogen formation, though it only occurred when at least 0.5 p.c. sugar was present in the perfusing blood.

In our experiments we have usually found a small glycogen formation in the livers of cats, and have occasionally observed it in those of dogs, although initially no glycogen was present, and although the blood sugar was not greater than 0.2-0.4 p.c. Thus, out of six experiments on cat livers, an amount of glycogen between 0.10 and 0.23 p.c. was found at the end in five cases.

The Origin of the Sugar

Lactic acid It has been shown by Baldes and Silberstein⁽¹⁷⁾ that in the perfused liver of the phloridzinised dog, sugar may make its appearance coincidently with the disappearance of an amount of lactic acid enough to account for the sugar. We have, therefore, determined the changes in the amount of lactic acid in the blood during the course of liver perfusions.

Method of estimation The lactic acid was estimated in the Folin-Wu blood filtrate, by the von Furth-Charnass oxidation method, after removal of the sugar and other interfering substances by means of copper sulphate and lime, as described by Clausen⁽¹⁸⁾.

The method consists, briefly, in oxidising the lactic acid in the

acidified solution at the temperature of the boiling water-bath with $N/200$ potassium permanganate, the acetaldehyde formed being caught in a solution of sodium bisulphite

After destruction of the excess of bisulphite with iodine solution, saturated sodium bicarbonate solution is added, and the bound bisulphite titrated with $N/200$ iodine

The accuracy of the method was considerably increased by the use of a new form of micro-aeration apparatus, employing absorption tubes of special construction which enabled the total volume of absorbing liquid to be reduced to 4 c.c., so that $N/200$ iodine could be used for the titration. Each c.c. of this iodine solution was taken to represent 0.25 mg. of lactic acid.

It is hoped shortly to publish a description of the apparatus in connection with a modified method for the determination of lactic acid.

Results

Exp 5 Dog Fat diet 7 days

Time	Blood sugar p.c.	Lactic acid mg per 100 c.c.
After 5 mins. perfusion	0.200	31.5
35 "	0.332	24.0
65 "	0.382	27.5

Exp 6 Cat Pancreas removed 3 days before

Time	Blood sugar p.c.	Lactic acid mg per 100 c.c.
After 5 mins. perfusion	0.271	43.5
35 "	0.364	44.0

Exp 7 Dog Fat diet 16 days

Time	Blood sugar p.c.	Lactic acid mg per 100 c.c.
After 5 mins. perfusion	0.176	71.5
35 "	0.264	53.5
65 "	0.302	35.0
95 "	0.340	35.0
125 "	0.382	34.5

These experiments all show that the production of sugar is in no way accounted for by a disappearance of lactic acid, in Exps 5 and 6 the lactic acid changes are trivial or absent, in Exp 7 there was indeed a disappearance at the rate of 18 mg per 100 c.c. during the first two half-hour periods, but the evidence suggests that this was not due to a conversion into sugar. The sugar production was the same during the second, third and subsequent periods, so that the origin of the sugar was presumably the same throughout this time, since during the third

Second experiment

Time	Rise in reducing power expressed in mg per 100 c c blood	
	Shaffer Hartmann	Hagedorn Jensen
3rd half hour	38	45
4th "	42	42
5th } "	80	83
6th }		
	160	170

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and fourth periods there was no change in lactic acid, it seems fair to suppose that the diminution in the second period was unconnected with the sugar formation

Relation of sugar to urea and ammonia nitrogen At this stage it seemed likely that the sugar produced originated from protein undergoing breakdown in the course of the perfusion. To investigate this possibility, determinations were made of the rise of urea and ammonia nitrogen side by side with determinations of the sugar

If all the carbon in the protein molecule be re-combined so as to form dextrose, the ratio of dextrose produced to nitrogen set free in the form of urea and ammonia cannot be greater than 8.3. The highest values ever observed by Lusk are in the neighbourhood of 4.0. In our earlier experiments we determined the nitrogen both in liver and in the blood at the beginning and at the end, arriving at figures for the initial and final total. The results showed that the percentage of urea and ammonia nitrogen in the blood, once the perfusion had begun, could be taken to represent the percentage in the system of liver and blood, just as in the case of sugar. Accordingly, in the later experiments we confined our attention to the estimation of both sugar and nitrogen in blood samples withdrawn from the apparatus at different stages of an experiment

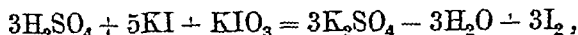
Methods The method which we employed was substantially that of Folin and Wu (19), but one or two modifications should be noted

Firstly, the use of the micro-aeration apparatus previously mentioned, for the removal and collection of the ammonia formed by digestion with urease, permitted the use of small quantities of blood for the determination. Thus, from 1 to 5 c.c. of the Folin-Wu blood filtrate was placed in the reaction flask, together with sufficient water to make a total volume of 5 c.c., and then two drops of sodium pyrophosphate solution and 1 c.c. urease solution (freshly prepared from soy-bean meal in accordance with Folin's directions) were added. The flask was then connected with the apparatus and immersed in a beaker of water at 40° C. for 15 to 20 minutes, after which the ammonia was liberated by the addition of 2 c.c. of a saturated solution of potassium carbonate, and carried over by a current of ammonia-free air, into the two absorption tubes, each containing 2 c.c. of *N*/100 sulphuric acid. During the aeration, which lasted for half an hour, the water in the beaker was kept gently boiling.

The principal objection to the usual method of titrating with alkali the excess of acid in the absorption tubes is the difficulty of obtaining a sharp end-point with the usual indicators. Advantage was therefore

taken of the fact that in the presence of an excess of potassium iodide and of potassium iodate, the free acid liberates hydriodic and iodic acids, which interact with the formation of iodine. Thus, three drops each of saturated solutions of potassium iodide and iodate were added to each tube, and the liberated iodine titrated with $N/200$ sodium thio-sulphate and starch solution.

The reaction may be formulated thus



from which it is seen that the excess of acid liberates an equivalent amount of iodine. The acid neutralised by the ammonia is then obtained in the usual manner by subtracting the titration figure from the amount of acid employed (expressed in terms of $N/200$ thiosulphate), namely, 4 c.c., and each c.c. of this represents 0.07 mg. nitrogen. A blank experiment should be carried through, as the urease solution may contain appreciable quantities of ammonia.

By this method 0.1 mg. of nitrogen, i.e. the amount normally present in $\frac{1}{2}$ c.c. of blood, may be estimated within an error of about 5 p.c.

The estimations of ammonia and urea nitrogen in the liver were carried out on portions of the protein-free solution as used for estimation of the sugar, and call for no special comment.

Special attention was paid to keeping all apparatus free from traces of heavy metals, since it was found that these rapidly destroyed the activity of the urease.

The following results were obtained in an earlier experiment

28 VII 25 Perfusion of liver of fat-fed cat

1 Urea and ammonia nitrogen

(a) In original blood 20.65 mg. per 100 c.c.

In liver sample 32.00 " "

Original blood was 245 c.c. in amount, while liver perfused weighed 55 gm. Hence total $N = 50.6 - 17.6 = 68.2$ mg.

(b) In blood after 5 minutes' perfusion there were 24.1 mg. per 100 c.c. Reckoning this as evenly distributed in the combined weight of liver and blood, i.e. in 300 gm., the total is 72.3 mg. The two figures for the initial total thus agree closely.

(c) In blood after 4 hours' perfusion 36.95 mg. per 100 c.c. Reckoning this as distributed in the liver and blood, i.e. in 300 gm., the total is 110.8 mg. Hence rise in nitrogen = 42.6 mg. The rise in sugar during the perfusion was from 90.7 mg. to 136.0 mg., i.e. 45.3 mg. Hence the ratio of $\frac{\text{dextrose}}{\text{nitrogen}}$ was $\frac{45.3}{42.6} = 10.6$

Subsequently we compared the rise of urea and ammonia nitrogen in blood samples withdrawn during perfusion with the rise in blood sugar. We were careful in all experiments to determine the total carbohydrate in the system before the perfusion began, so that we knew with certainty that the rapid rise of sugar in the first half-hour was due to fresh formation and not to washing out from the liver.

The following is an example

Exp 8 Dog on fat diet 7 days

The liver sample contained no glycogen. Total carbohydrate in system

By method (1) (see p 504) 746 mg

By method (2) 831 mg

The agreement between these figures indicates that they are approximately correct, and that the rise of blood sugar after 35 minutes is a production of sugar *de novo*.

Time	Blood sugar mg per 100 c.c.	Blood nitrogen mg per 100 c.c.	
		(a)	(b)
After 5 minutes	109	9.5	9.5
35 "	220	12.3	12.8
65 "	268	14.5	15.5
95 "	268	17.3	18.3

During first half-hour the value of $\frac{\text{dextrose}}{\text{nitrogen}}$ is $\frac{111}{31} = 3.58$, and during the second half-hour the value is $\frac{48}{2.4} = 20$. During the third half-hour the value is 0, for no rise of sugar took place, on the other hand, the rise of nitrogen was similar to that in the second and first half-hours.

The remainder of the results which we have obtained in these ways (with the exception of three to be discussed in the next section) are summarised in Table II.

TABLE II

A	Animal	Period	D/N	D/N ratio in half hour periods				
				1st	2nd	3rd	4th	5th
<i>Exp 19</i>	Cat	3 hours	8.7					
	"	80 minutes	10.3					
<i>B</i>	Dog							
				17.5	5.8	2.0	12.0	14.7
				9.0	4.0	—	—	—
				9.7	6.2	—	—	—
				9.6	7.2	9.2	—	—
				14.6	4.6	2.2	—	—
				45.0	14.6	4.0	19.1	—
				19.4	11.4	10.7	—	—
				25.0	0.6	7.8	—	—
				12.6	3.0	6.4	—	—
				17.0	6.8	8.4	—	—

If we add to the figures in Table II those obtained in experiments described in detail above and hereafter, a total of 47 determinations of a D/N ratio have been made, of which 32 exceed the value 8.3. In seven cases the ratio has exceeded the value 17. The results display no direct relationship of any kind between the rise of reducing power of the blood and that of the urea and ammonia nitrogen, indeed, there were cases where it seemed that an inverse relationship might exist. In Exp. 9 in Table II in which ratios for five half-hour periods are shown, the low ratio in the third period was due not only to a smaller sugar production, but to a greater nitrogen production than before or later, this was also the case in Exp. 14 of Table II.

In three experiments not recorded above we have obtained figures for (a) the rise of blood sugar, (b) the rise in urea and ammonia nitrogen, (c) the lactic acid content of the blood.

Exp. 19. Dog 7 kgm. On fat diet 8 days

Initial carbohydrate in system by first method, 1046 mg

Initial carbohydrate in system by second method, 1014 mg

Time	Sugar	Nitrogen in mg per 100 c.c.	Lactic acid
After 5 mins. perfusion	200	12.0	31.5
35 " "	332	21.1	24.0
65 " "	382	27.0	27.5

In the period from 5 to 35 minutes, 7.5 mg of lactic acid disappeared. If it is assumed that this was converted to sugar, the production of sugar from other sources was 124.5 mg. The ratio of this to the nitrogen rise of 9.1 mg gives the value 13.6. In the period 35 to 65 minutes there was no disappearance of lactic acid, and the relation of sugar to nitrogen is 8.4.

Exp. 20. Dog 9 kgm. On fat diet 15 days

Time	Sugar	Nitrogen in mg per 100 c.c. blood	Lactic acid
After 5 mins. perfusion	176	9.4	71.5
35 " "	264	14.85	53.5
65 " "	302	20.35	35.0
95 " "	340	25.15	35.0
125 " "	382	27.85	34.5
185 " "	462	35.20	—

In the period 5 to 35 minutes, 18.0 mg lactic acid disappeared. If we assume this converted to sugar, then the production of sugar from other sources is 70 mg. The ratio of this to the rise in N is 12.8. In the second period, a similar calculation gives $D/N = 3.6$, in the third period $D/N = 8.0$, and in the fourth period $D/N = 15.3$.

In this experiment the sugar determinations were done by two methods, each method being used to obtain four estimations of each sample, the lactic acid and nitrogen figures were obtained in duplicate, the agreement between duplicates being close in all cases. The sugar production persisted at a uniform rate for $2\frac{1}{2}$ hours after the end of the first half-hour perfusion, and no sign of oedema was observed in the liver. Consequently we attach considerable weight to the figure of 15.3 as expressing the ratio of sugar to nitrogen produced during the fourth period in which there was no change in lactic acid.

Finally, we have carried out one experiment on the liver from a depancreatized cat. In this case, too, we have figures for sugar, nitrogen and lactic acid during the first half-hour's perfusion. During this time there was no change in lactic acid, while the relation of sugar to nitrogen was 11.2.

The production of acetone bodies In all experiments a large production of acetone bodies was observed. The estimation was carried out by the method of van Slyke and Fitz(20), the precipitate of the mercury acetone compound being filtered through a Gooch crucible and weighed. We found the method quite satisfactory, though Laufberger(21) says that he obtained negative results. Table III shows the extent of the formation, and that the production was not obviously influenced by the presence of insulin. Laufberger(21) states that the insulin prevents acetone body formation in the perfused liver, he was not, however, perfusing the livers of fat-fed animals. We propose to investigate further the relationship between acetone body formation and sugar production.

TABLE III

Exp	Time	Acetone bodies reckoned as β hydroxy butyric acid in mg per 100 c c	Dextrose in mg per 100 c c.	Hormone
				None
Exp	After 5 mins. perfusion	Trace	62	—
	35 "	48	135	
	65 "	95	174	
Exp	After 5 mins perfusion	13	79	20 units insulin
	35 "	49	132	
	65 "	100	164	

Discussion

In the experiments described we have repeated the observation of Embden that a liver removed from the body and perfused with blood from the same species produces sugar in important amounts, we have found that the rate varies from 2 to 4 mg sugar per gram of liver per hour.

In Embden's experiments this production ceased after a short interval, though it could be renewed by replacing the blood with a fresh lot. While we found a similar cessation of production in many cases, this was not invariable, and in those cases where the conditions of perfusion were the best, the sugar production was most prolonged, we came to the conclusion that the cessation of sugar production observed by Embden was an indication, not that the source was exhausted, but that damage to the liver cells had occurred. We are inclined to think that an important addition to the blood in a liver perfusion is a certain small concentration of adrenaline, this was present in one experiment in which a steady sugar increase was observed for 3 hours, its importance in maintaining proper circulatory conditions in the liver capillaries may be the explanation of the renewed sugar production in Embden's experiments when a fresh lot of defibrinated blood was used.

Our experiments differ from those of Embden in that we obtained livers initially almost carbohydrate-free by feeding the animals on an exclusively fat diet. Moreover, we have been able to show that the sugar formed does not originate from lactic acid as Bornstein and Griesbach(4) have suggested, and further that a large part of it at least cannot come from protein. A consideration of our technique makes it unlikely that the sugar comes from any diffusible constituent of the liver initially present such as glycollic aldehyde(22) or di-oxyacetone(23), because it was our practice to wash out all such substances with a portion of blood, which was allowed to perfuse the liver once, and was then discarded. The success attending this procedure appears from the fact that in early cases, in which it was not carried out, the percentage of free sugar in the liver was 1.1-1.4, while in all later experiments, the washing through reduced this concentration to about 0.1 p.c.

It seems to us that these observations must be considered in forming any opinion on the much-debated question of whether carbohydrate can be formed from fat. It was impossible in our experiments to obtain evidence that there was such a disappearance of fat as would account for the sugar found, because the total of fat was so large and the percentage in different lobes of the liver varied considerably. Nevertheless we know of no other source from which the sugar could have come.

It may be significant that our results with the livers of fat-fed animals resemble closely the result on the one liver we took from a depancreatized animal. There was the same sugar production, unaccounted for by changes in lactic acid content or by appearance of urea and ammonia nitrogen in both cases. Geelmuyden(24) has already suggested

that the chief error of metabolism in diabetes is a continuous overproduction of sugar by the liver, following this it is easy to imagine that we have in these experiments a demonstration of what is taking place in the body when the carbohydrate economy is upset by a lack of either carbohydrate or of the pancreatic hormone

Our experiments in which insulin was added to the blood did not, indeed, give evidence of an inhibiting action on the new formation of carbohydrate. This failure, however, can hardly be held to weaken the evidence of this action from experiments of other kinds. The production of carbohydrate varied so much, and tended so often to decline during the course of an experiment when nothing was added to the blood, that it was not possible to predict with certainty, in any one instance, the course of events if insulin had not been added. The failure to demonstrate this effect is comparable to that of most observers who have endeavoured to detect, in experiments on the perfused liver, the glycogen storage which insulin undoubtedly promotes in the diabetic animal

SUMMARY

1 When a liver taken from a cat or dog fed on a fat diet is perfused with blood from the same species there is a production of reducing sugar at a rate of from 20 to 40 mg per gram of liver per hour. A small formation of glycogen is demonstrable

2 This sugar does not come from lactic acid

3 Determination of the ratio of sugar produced to nitrogen produced in the form of urea and ammonia shows that only a small fraction of the sugar can come from protein

4 The procedure adopted makes it improbable that the sugar originates from any diffusible constituent of the liver

5 The production of sugar is not obviously influenced by insulin, adrenaline or pituitary extract

6 One liver taken from a depancreatized cat gave a similar result

7 The perfused livers usually produced a steady secretion of bile

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THE PERIPHERAL INNERVATION OF THE VESSELS OF THE EXTERNAL EAR OF THE RABBIT

By W FELDBERG (*Berlin*)

(*From the Physiological Laboratory, Cambridge*)

ALTHOUGH the demonstration of the alteration of the calibre of the vessels in the rabbit's ear furnished one of the earliest proofs of the existence of vaso-constrictor fibres, there still remains considerable uncertainty as to the precise nervous supply of the vessels of this organ. The object of the present research was to clear up these uncertainties as far as possible, both with regard to vaso-constrictor and vaso dilator fibres.

The external ear is provided with nerves from the V and VII cranial nerve and with nerves from the spinal cord as follows

(1) The facialis supplies the ear muscles, some branches of which leave the Fallopiian canal before reaching its termination, these are known as the facialis posterior

(2) The trigeminus gives to the ear the ramus auricularis temporalis

(3) Nerves from the II and III cervical form the great auricular (or auricularis anterior) and the posterior auricular. The sensory nerve supply of the ear consists of these fibres with the addition of the auriculo temporal branch of the trigeminus

Langley has suggested that the anterior auricular should be called the ventral auricular and similarly, that the posterior auricular should be called the dorsal auricular. His reason was that the anterior auricular supplies the posterior part of the ear, and the posterior auricular supplies the anterior part of the ear. They were given their names because the anterior (ventral) auricular came chiefly from the anterior (ventral) branches of the II and III cervical and the posterior (dorsal) auricular from the posterior (dorsal) branch of the II cervical. In this paper we shall use the terminology suggested by Langley¹

We could not find that the first cervical nerve sends any fibres to the ear

¹ Shortly before his death, Prof. Langley worked on the problem of the peripheral innervation of the vessels of the rabbit's ear. He intended that his work and mine should be published together. After his death I finished the experiments alone

Method The animals were in all cases anæsthetised and tracheotomised. We observed the vessels with the naked eye by transmitted light—usually electric light but occasionally daylight. In all cases we were careful to eliminate the factor of warmth.

We employed both electrical and mechanical stimulation, usually the former. Mechanical stimulation was simply made by compressing the nerve with forceps for either 10–20 sec. or else for several short intervals.

The extirpation of the superior cervical ganglion This was made under chloroform-ether anæsthesia. The dilatation of the ear vessels after the extirpation of the ganglion disappeared in a few days. Even the next day only the central artery showed a small dilatation especially in its lower part. Under normal conditions this dilatation disappeared also in a few days. In the few cases, where the wound took some time to heal, the central artery remained dilated for a longer time. Similar observations on the denervated hind-legs are described by Goltz⁽¹⁾.

A fortnight to two months elapsed between the extirpation of the ganglion and the observation of the effect. During the process of cutting the hair on the operated and normal sides respectively it was noticed that the manipulation due to this procedure made the ear red more rapidly on the operated side than on the control, and the red colour was longer maintained. Later on, when we began the chloroform-ether anæsthesia (the rabbits were put under a glass bell) the ear vessels of the operated side dilated more quickly than the vessels on the normal side. They remained also longer dilated after the administration of the anæsthetic was stopped. On several occasions no dilatation on the sound side took place if the anæsthetic was removed at the moment when the vessels on the operated side became dilated. There appears to be a contrast between the effects of chloroform-ether and those of amyl nitrite (Eugling⁽²⁾).

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THE PERIPHERAL INNERVATION OF THE VESSELS OF THE EXTERNAL EAR OF THE RABBIT

BY W. FELDBERG (*Berlin*)

(*From the Physiological Laboratory, Cambridge*)

ALTHOUGH the demonstration of the alteration of the calibre of the vessels in the rabbit's ear furnished one of the earliest proofs of the existence of vaso-constrictor fibres, there still remains considerable uncertainty as to the precise nervous supply of the vessels of this organ. The object of the present research was to clear up these uncertainties as far as possible, both with regard to vaso-constrictor and vaso dilator fibres.

The external ear is provided with nerves from the V and VII cranial nerve and with nerves from the spinal cord as follows

(1) The *facialis* supplies the ear muscles, some branches of which leave the Fallopiian canal before reaching its termination, these are known as the *facialis posterior*

(2) The *trigeminus* gives to the ear the *ramus auricularis temporalis*

(3) Nerves from the II and III cervical form the great auricular (or *auricularis anterior*) and the posterior auricular. The sensory nerve supply of the ear consists of these fibres with the addition of the *auriculo temporal* branch of the *trigeminus*

Langley has suggested that the anterior auricular should be called the ventral auricular and similarly, that the posterior auricular should be called the dorsal auricular. His reason was that the anterior auricular supplies the posterior part of the ear, and the posterior auricular supplies the anterior part of the ear. They were given their names because the anterior (ventral) auricular came chiefly from the anterior (ventral) branches of the II and III cervical and the posterior (dorsal) auricular from the posterior (dorsal) branch of the II cervical. In this paper we shall use the terminology suggested by Langley¹

We could not find that the first cervical nerve sends any fibres to the ear

¹ Shortly before his death, Prof. Langley worked on the problem of the peripheral innervation of the vessels of the rabbit's ear. He intended that his work and mine should be published together. After his death I finished the experiments alone.

Method The animals were in all cases anaesthetised and tracheotomised. We observed the vessels with the naked eye by transmitted light—usually electric light but occasionally daylight. In all cases we were careful to eliminate the factor of warmth.

We employed both electrical and mechanical stimulation, usually the former. Mechanical stimulation was simply made by compressing the nerve with forceps for either 10–20 sec. or else for several short intervals.

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of the ganglion separately before drawing it out. The exact course of the operation was as follows

One hour and a half before the commencement of the operation 90-100 mg luminal were injected subcutaneously so that but little chloroform ether was needed. The rabbit lay on its side. The skin was cut between the dorsal edge of the scapula and the spinal vertebrae, the front end of the incision reaching some cm above the anterior border of the scapula. The slender transversus muscle was cut. The scapula with its muscles was raised a little and drawn down, so that the upper part of the chest lay free. The scapula was held in its position by a hook. The first rib is covered by the musculus scalenus medius, which was cut at this level. Under this muscle lies the small musculus scalenus anterior which was gently torn across. The roots of the brachial plexus were now exposed. We dissected deeper with a small forceps between the first two branches of the plexus, which issue in front of the first rib and join a little later to form a triangle. The upper part of the ganglion stellatum was then always found at once by keeping quite near to the first rib and not too ventral. Each fibre of the ganglion was separately cut through, first the upper branches, then slowly drawing out the ganglion we cut the rest one fibre after the other. At last it tore off, usually with a small piece of the sympathetic remaining on the lower end. We sewed the musculus scalenus medius and transversus with two sutures and then the skin. The operations were made under strict asepsis. The animals always recovered very quickly apart from a slight limp of the fore leg on the operated side.

The post-mortem examination showed that the ganglion was always completely removed and that the wound had thoroughly healed. We made in all five operations on the right side and two operations on the left side.

After the removal of both the superior cervical ganglion and stellatum on one side, the vessels did not return to their original degree of contraction. Even four weeks after the operation the vessels showed a small but distinct dilatation compared with the vessels of the normal side. The same difference in the calibre of the vessels was found in two of the three experiments, in which only the ganglion stellatum had been removed. This is not the case after the extirpation of the superior cervical ganglion. It should be remembered, however, that the extirpation of the ganglion stellatum also removes the whole central supply of the superior cervical ganglion, so that the influence from the central nervous system on the constrictor tonus of the ear vessels remains. This incomplete return of tonus agrees with isolated observations of Eugling⁽²⁾ and with those of Krogh⁽⁵⁾ on the capillaries.

The preparation of the auriculo temporalis (V) In the dead rabbit this rather thin nerve is best found at its origin, where it leaves the trunk of the maxillaris inferior. From here it comes up towards the surface behind the posterior border of the ramus mandibularis of the mandible and soon divides into its branches to the facialis, to the regio temporalis and to the ear. In one animal the nerve was still divided at its origin.

The preparation of this nerve in the living animal is easy if the rabbits used are not too fat and muscular. The most suitable are young female animals. The skin and fascia were cut between the base of the ear and the zygoma. We dissected behind the posterior border of the mandibularis ascendens close to the border of the medial part of the masseter muscle and reached the vena facialis posterior. In front of this vein we went deeper. The branch to the ear runs under and behind the vein. The trunk of the nerve is enclosed with the pterygoid muscle above, the fat of Bichat below, the ramus mandibularis ascendens in front and the tympanic bulla behind. The nerve was freely exposed, tied and cut as deeply as possible.

The preparation of the auricularis ventralis and dorsalis and of the facialis posterior. The skin was cut 2 cm. from the basis of the ear obliquely to the neck. After cutting the fascia the auricularis ventralis was to be found behind the vena auricularis posterior. The nerve comes out behind the posterior border of the musculus sternocleidomastoideus and runs in an oblique direction to the base of the external ear. To find the auricularis dorsalis we went towards the back and under the two next muscles. The branches of the facialis posterior were found between both the auricular nerves on the base of the ear.

THE PERIPHERAL DISTRIBUTION OF THE VASO-CONSTRICTORS OF THE EAR

The vaso-constrictor fibres run in the nervi facialis posterior, auricularis ventralis and auricularis dorsalis. We could not confirm the observation of Lowen⁽⁶⁾ that fibres run directly from the superior cervical ganglion in close connection with the arteries which distribute to the ear, for after having cut both the cervical nerves and all branches of the facialis posterior, stimulation of the cervical sympathetic did not cause the slightest constriction of the vessels of the ear.

The nerves each supply special parts of the ear with vaso-constrictor fibres. These areas overlap, particularly those supplied by the facialis and the cervical nerves. We may leave out of consideration the individual variations because they do not alter the nature of the distribution.

The auricularis ventralis. Cutting this nerve always caused a marked dilatation of the ear. The dilatation took place chiefly on the distal part and the posterior border or the side of the ear. The basal part of the central artery was only slightly dilated as also the anterior border of the ear.

Very strong currents were needed to give a vaso-constriction on

stimulating this nerve This was pointed out first by Morat and is due to the thickness of the sheaths of this nerve (Fletcher(7)) In our experiments the secondary coil was between 10-6 cm , at 7-10 cm the current was unbearable on the tongue The constriction took place chiefly on the distal part and posterior border of the ear The anterior border was only contracted on the tip (see Fig 1) The basal part of the central artery was only weakly affected even with very strong current From 5 to 15 sec , after ceasing the stimulation, an after-flush occurred This gradually increased and faded slowly after some minutes

Similar observations about the distribution of this nerve were made by Dastre and Morat(8), Fletcher(7), Langley(9), Lowen(6) and Schiff (quoted by Langley) Fletcher, however, states that he also found "the proximal third of the artery to show marked constriction equal in one case to that in the distal third "

Auricularis dorsalis Cutting this nerve always caused a strong dilatation on the anterior border of the ear, the electrical stimulation caused a strong constriction in this region (see Fig 3) If the ventral auricular had been cut previously, the contracted vessels were sharply marked against the remaining dilated ear

Facialis posterior The cutting of all the branches of this nerve caused a marked dilatation of the proximal part of the ear

Electrical stimulation of this nerve always caused a marked constriction of the same region It is impossible to stimulate all branches of this nerve together In order to observe the exact distribution of the whole nerve, we stimulated the peripheral end of the cervical sympathetic after having cut previously the ventral and dorsal auricular The facialis posterior receives, as we shall see later, all its vaso-constrictor fibres from the superior cervical ganglion, so that stimulation of the sympathetic nerve involves all the fibres of the facialis posterior, and these fibres only, since the cervical nerves are cut Under this condition the stimulation of the cervical sympathetic caused a contraction of the proximal half of the ear, the contracted part reaching higher in the middle of the ear than on both sides (see Fig 5)

THE ORIGIN OF THE POST-GANGLIONIC FIBRES OF THE VASO-CONSTRICTORS OF THE EXTERNAL EAR

Fletcher's(7) work showed that the ventral auricular obtains vaso-constrictor fibres from the ganglion stellatum, but did not make it clear whether all its vaso-constrictor fibres have this origin, and Fletcher did not deal with the question whether the dorsal auricular, which

supplies only the small anterior part of the ear, obtains any fibres from the ganglion stellatum

In order to ascertain which nerves receive fibres from the superior cervical ganglion, we cut all the nerves of the ear, except the one we wished to examine, and then stimulated the peripheral end of the cut cervical sympathetic. The contraction which resulted and which was abolished by cutting the remaining nerve could only be due to fibres from the superior cervical ganglion running to this nerve. The second method used was the excision of the ganglion stellatum and the stimulation of the ear nerves after allowing time for degeneration.

To see which nerves obtain fibres from the ganglion stellatum we removed the superior cervical ganglion and waited the time necessary for degeneration. The constriction now obtained on stimulating the ear nerves was due to fibres from the ganglion stellatum, since, after having removed both ganglia, stimulation of the ear nerves never gave the slightest vaso-constriction. The results in the special nerves were the following.

Facialis posterior This nerve receives all its vaso-constrictor fibres from the superior cervical ganglion, because, after having removed this ganglion and waited the time necessary for degeneration, stimulation of this nerve did not cause a constriction. On the other hand, after having removed the ganglion stellatum the vaso-constriction on stimulating this nerve was not diminished.

Auricularis ventralis This nerve receives fibres from both ganglia, as is shown by the following results.

(1) Stimulation of the peripheral end of the cervical sympathetic (the dorsal cervical and all branches of the facialis posterior being cut previously) caused a constriction, chiefly for about 2 cm. in the middle of the central artery of the ear. The remaining upper half of the artery was only slightly contracted but many of the smaller vessels had disappeared. After cutting the ventral auricular as well, the stimulation of the cervical sympathetic did not cause a constriction.

(2) After the excision of the ganglion stellatum (three experiments) stimulation of the ventral auricular nerve still caused a constriction. The central artery showed the best constriction in the middle of the ear. The part corresponded well with that on stimulating the cervical sympathetic under the condition in para 1 (*supra*). In comparison with the contraction obtained on the normal side the effect was always smaller, in particular the tip and the posterior border remained more or less dilated (see Fig 2).

This agrees with our observation on cutting the cervical sympathetic. In some experiments the posterior border remained contracted and the tip was little dilated, in the other cases the whole ear dilated equally (see also Tigerstedt(10))

These two series of experiments showed that the ventral auricular receives some fibres from the superior cervical ganglion

(3) After having removed the superior cervical ganglion and having waited the time for degeneration, stimulation of the ventral auricular with strong currents always caused a constriction (Schiff, Fletcher). Weak currents often produced vaso-dilatation. This will be discussed in the section on the vaso-dilator nerves

The contraction was mostly stronger than that after the ganglion stellatum had been removed, so that it seemed that the ventral auricular receives more fibres from the ganglion stellatum than from the superior cervical ganglion

(4) After having removed both the ganglia, stimulation of the ventral auricular did not cause any contraction even with the strongest currents

Auricularis dorsalis The dorsal auricular obtains most of its fibres from the superior cervical ganglion. We found, however, that after removing this ganglion we were able to cause a small constriction on the upper part of the anterior border in all experiments. This constriction concerned only a very small region in comparison with that obtained on the normal side (see Fig 4). As it was abolished after excision of both the ganglia it indicates the origin of some fibres from the ganglion stellatum. Stimulation of the dorsal auricular after the excision of the ganglion stellatum did not show a distinct diminution of the constriction. We may conclude, therefore, that this nerve receives most of its fibres from the superior cervical ganglion and only some from the ganglion stellatum. These latter explain the non-degenerate fibres on the anterior border of the ear, which Eugling found constantly after having removed the superior cervical ganglion and cut the ventral auricular previously

In the dog and in the cat the vessels of the ear are not provided with any post-ganglionic fibres from the ganglion stellatum. Fletcher, since he found only fibres from the ganglion stellatum in the *auricularis ventralis*, legitimately assumed the reason to be that this nerve is more anterior in the cat than in the rabbit. We, however, have found fibres from the ganglion stellatum in both cervical nerves and our explanation is that even the pre-ganglionic fibres for the ear vessels are more anterior

in origin in the cat than in the rabbit. As Langley(11) showed, the pre-ganghonic fibres for the ear vessels run in the cat in the I-IV thoracic nerves and in the rabbit in the II-VIII thoracic nerves.

We must furthermore assume that the ramus vertebralis in the rabbit sends vaso-constrictor fibres not only to the third but even to the second cervical nerve.

The figures show diagrammatically the vaso-constrictor distribution in the external ear of the rabbit. The shaded sections are the parts in which contracted vaso-constriction occurs on stimulation of the nerves.

The ears are seen from behind so that the anterior border becomes the medial, the posterior the lateral border of the ear.

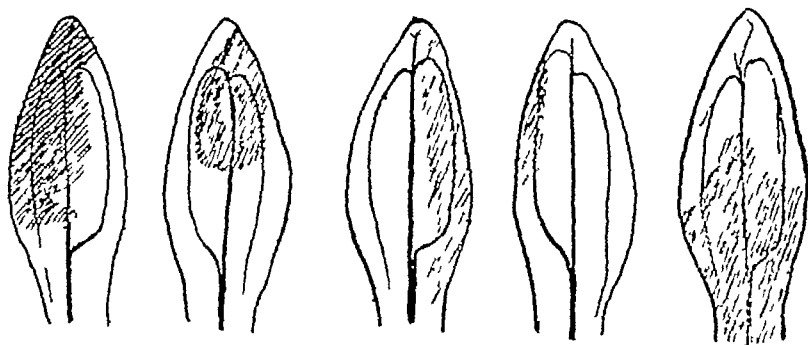


Fig 1

Fig 2

Fig 3

Fig 4.

Fig 5

Fig 1 Left ear. Contraction due to stimulation of the ventral auricular (p. 522)

Fig 2 Right ear. The same as in Fig 1, only the ganglion stell. removed three weeks previously. In the contracted area a small part of the central artery remained dilated (p. 523)

Fig 3 Left ear. Contraction due to stimulation of the dorsal auricular (p. 522)

Fig 4 Right ear. The same as in Fig 3, only the sup. cerv. ganglion removed three weeks previously (p. 524)

Fig 5 Left ear. Contraction due to stimulation of the periph. end of the cut cerv. sympathetic. The auriculo-cervical nerves are cut previously. The figure shows the distribution of the vaso-constrictors of the facialis posterior (p. 522)

THE VASO-DILATORS OF THE EXTERNAL EAR

As to the origin of the vaso-dilators of the external ear of the rabbit, we find in the literature several statements which we can divide into three groups

(1) The fibres in the cervical sympathetic

Dziedzuhl(12) found that four days after the section of the cervical sympathetic stimulation of this nerve caused a vaso-dilatation of the ear

vessels It could be obtained till the eleventh day after the section Dziedzuhl's explanation was, that the vaso-constrictor fibres of the cervical sympathetic degenerate more quickly than the vaso dilator fibres, which under normal conditions are masked and which now appear The experiments were recently repeated by Feldberg and Schilf⁽¹³⁾, who could not confirm the results References to earlier work on the subject are given in their paper

(2) The vaso-dilatation of the auriculo temporalis (V)

Schiff⁽¹⁴⁾ succeeded in obtaining a vaso-dilatation of the ear vessels in six out of 11 experiments, when he stimulated the intact auriculo temporalis or its peripheral end The vaso-dilatation began at once with the stimulation and ceased with it Vulpian⁽¹⁵⁾ could not confirm this, Claude Bernhard⁽¹⁶⁾, however, sometimes observed on the ear of the dog a vaso-dilatation on stimulating this nerve

(3) The "antidromic" vaso-dilatation and the dilatation of the cervical auricular nerves

Krogh and Rehberg⁽¹⁷⁾ tried vainly "to elicit any dilator response by electrical or mechanical stimulation of the peripheral ends of the sensory nerves to the rabbit's ear" But when they observed the vessels microscopically and stimulated them directly with a fine needle or a hair, they obtained a local vaso-dilatation which disappeared gradually with the proceeding degeneration of the cut cervical nerves Nineteen days after the section it could no more be produced

Winkler⁽¹⁸⁾ observed a vaso-dilatation of the ear after section of the dorsal (and ventral) roots of the IV, V and VI cervical nerves Whether this vaso-dilatation can be regarded as an antidromic vaso dilatation, as Bayliss believed, or more properly as a reflex one, is not proved The sensory cervical nerves which supply the ear come from the II and III cervical nerves, but the possibility of some kind of antidromic dilatation must be taken into account

We made experiments on the auriculo-temporalis (V) and on the two cervical nerves

Auriculo-temporalis (V) Our experiments were made on ten rabbits In two of the animals the superior cervical ganglion was removed previously We stimulated mechanically and electrically, always beginning with weak currents, gradually increasing In the first experiment we observed a vaso-dilatation of the central artery 5-25 sec after the electrical stimulation of the nerve had ceased This dilatation lasted some seconds only In all the following nine experiments, however, we did not succeed in obtaining a distinct vaso-dilatation During the first experiment we

were probably less alive than later to the rhythmic variations first observed by Schiff and to central influences. We also believe, that the vaso-dilatation of the six experiments of Schiff during the stimulation of the nerve was due to central influences. Schiff stimulated in some experiments the intact nerve with central connections undisturbed. The form of the vaso-dilatation described by him (the lack of a latent period, the beginning and ceasing with the stimulation) differs considerably from that always observed on stimulating vaso-dilators in sensory nerves.

The auricularis ventralis and dorsalis Before I began my experiments the late Prof. Langley wrote me, "that sometimes the cervical auricular nerves (chiefly the great auricular) under strong induction currents of low frequency or weak induction shocks of high frequency, caused a great dilatation of the vessels in the ear, gradually beginning in the capillary region." He advised me to take away the ganglion cervical superior or the ganglion stellatum to see if it would then be easier to obtain a vaso-dilatation on stimulating these nerves.

In four experiments, in which both the ganglia of one side were removed 11-25 days previously, mechanical and electrical stimulation with weak and strong currents of both the auriculo cervical nerves caused a marked dilatation. The dilatation of the ventral auricular concerned the whole ear except the anterior border. That of the dorsal auricular concerned the anterior border. The vaso-dilator distribution is the same as the sensory nerves distribution and nearly the same as that of the constrictor fibres.

The dilatation occurred on mechanical stimulation after a latent period of 10-25 sec. On electrical stimulation which lasted one minute it occurred during the stimulation with a weak current 25-30 sec, with a strong current 5-10 sec after the beginning of the stimulation. The dilatation began in the smallest vessels and spread gradually to the great vessels, including also the central artery when the ventral auricularis was stimulated. During the first minute this vaso-dilatation increased, after 4 minutes it faded slowly away. Even with the strongest unbearable currents it was at no time possible to obtain the slightest vaso-constriction.

The section of these nerves also produced a constant vaso-dilatation. The ear showed a much redder tone (due to dilatation of the small vessels) than the ear of the normal side, where the cervical sympathetic was cut. The effect must be regarded as due to stimulation of the nerves by the section. While the dilatation due to the sympathetic section did not fade away for a long time, the dilatation after cutting the cervical

nerves gradually diminished and after 10-15 min all the small vessels had disappeared and the large vessels were no more dilated than before the beginning of the experiment (see Method) In the experiments on those rabbits on which only the ganglion stellatum or only the cervical ganglion had been removed, stimulation of the cervical nerves mechanically or with weak currents caused a dilatation Stronger currents always gave a vaso-constriction, the details of which we have already described In some cases, where the superior cervical ganglion had been removed, the current on stimulating the ventral auricular was just strong enough to show the effect on both dilator and (remaining) constrictor fibres the central artery contracted partly, but the small vessels dilated and the ear tip remained very red Weakening the current during the stimulation abolished the constrictor effect on the central artery, strengthening the current caused a contraction of the small vessels as well

As the two cervical nerves of the ear are sensory nerves there can be no doubt that the vaso-dilatation is due to "antidromic" reaction We think, however, that the sensory dorsal roots of cervicals II and III must be responsible for this and that the vaso-dilatation of the experiments of Winkler⁽¹⁸⁾ (c IV, V, VI) is a reflex effect

CONCLUSION

(1) A method of removing the ganglion stellatum above the first rib in the rabbit is described

(2) It is found that the vaso-constrictor fibres for the external ear run in the facialis posterior, the auricularis ventralis and the auricularis dorsalis

The origin of the post-ganglionic vaso-constrictor fibres of the facialis posterior lies in the superior cervical ganglion, of the auricularis ventralis and dorsalis in both the superior cervical ganglion and the ganglion stellatum The dorsal auricular, however, obtains only very few fibres from the ganglion stellatum

(3) Vaso-dilator fibres of the external ear could not be found in the auriculo-temporalis (V) They run in the auricularis ventralis and dorsalis Each nerve supplies a special region similar to the sensory distribution of these auriculo-cervical nerves

I should like to record my indebtedness to the late Prof Langley for much useful advice at the commencement of this research, and my appreciation of the loss it sustained when his death deprived me of his criticism and encouragement

Prof Barcroft has generously helped me with the preparation of this paper, and I wish to thank him for his patience and kindness

My wife has assisted me throughout the course of the experiments described above, and I owe much to her constant help

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THE REACTION BETWEEN ACETYL CHOLINE AND MUSCLE CELLS By A J CLARK

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ACETYL CHOLINE is exceptionally well adapted for the study of the laws governing the reactions between drugs and cells, for the following reasons

(1) It produces graded reactions over a remarkably wide range of concentrations

(2) It affects a variety of tissues and produces different types of actions in different tissues

(3) It acts rapidly and its action is quickly and completely reversible, hence a large number of experiments can be performed on a single preparation

The action of acetyl choline hydrochloride was studied on both the isolated ventricle and rectus abdominis of *Rana temporaria*. The drug used came from three sources, namely, a sample kindly given me by Dr H H Dale, a preparation of Messrs British Drug Houses and a preparation of Messrs Kahlbaum. The three samples did not differ appreciably in strength.

Experiments on the heart Acetyl choline when it acts on the intact isolated frog's heart produces several actions. It diminishes the frequency of the sinus rhythm, diminishes the rate of conduction between the auricle and ventricle and decreases the force of contraction of the auricle and ventricle. In order to obtain a quantitative measure of the action of the drug it was obviously desirable to study one effect alone, and the effect chosen was the depression of the force of contraction of the ventricle. A longitudinal strip of ventricle was cut off, consisting of about one-third of the ventricle. It was suspended in a container holding 5 c.c. and was driven at a constant rate by break induction shocks. The usual frequency was 20 per minute. The stimulus was passed through the length of the strip to eliminate any effects due to alterations produced by the drug in conduction. In most cases the responses were recorded isometrically by a light lever attached to a strip of steel. The lever gave a deflection of 1 cm. when 2 gm. tension was applied, and the relation between excursion and tension was linear up

to 5 grm tension. The tensions recorded usually were less than this. In some cases an isotonic lever was used. This provided a more sensitive measure of very slight effects but did not give as accurate a measure of actions approaching maximal, that is, complete arrest.

The Ringer's fluid had the following percentage composition: NaCl 0.65, KCl 0.014, CaCl_2 anhyd. 0.024, NaHCO_3 0.05, pH 8.0. Air was bubbled through the container. I found that acetyl choline decomposed fairly rapidly in dilute solution, and particularly rapidly in the alkaline oxygenated Ringer's fluid, care therefore was taken to use freshly diluted drug. The drug was sometimes added to the fluid in the container and sometimes the final dilution was made outside and the fluid in the container changed.

Hearts showed great individual variation in their sensitivity to the drug, but the response of a single strip was fairly uniform. In particular the strips did not show any well-marked increase or decrease in sensitivity after prolonged isolation. The responses given during the first hour often were irregular but a heart after 24 hours isolation showed the same sensitivity as a heart after 1 hour's isolation.

I found that changes in pH from 8.0 to 7.0 produced no demonstrable variation in the response of the heart to acetyl choline. Andrus and Carter(1) found that the drug acted on the heart more powerfully at pH 7.0 than at pH 8.0, but it seems possible that their results may have been due to the drug being destroyed more rapidly in alkaline than in neutral solution. Since slight changes in reaction did not appear to produce a measurable effect bicarbonate Ringer's fluid was used in preference to fluid buffered with borates because the preparation contracted more vigorously with the former fluid.

Acetyl choline produces its maximal action on the heart sometimes in a few seconds and always within a minute. After this the heart partially recovers, but the degree of recovery is very variable, as is shown in Fig. 1. A similar effect was noted by Straub(2) when muscarine acted on the heart. The significance of this effect will be discussed later.



Fig. 1. Partial recovery of heart during exposure to acetyl choline. The upper and lower curves are from experiments on different hearts. In both cases acetyl choline 10^{-4} molar was introduced at the arrow, and the second portions of the curves show the actions after exposure to the drug for 30 minutes.

This recovery effect is a source of error in quantitative experiments

I took the greatest effect produced by the drug as the measure of its action and in cases where the immediate effect was followed by a sharp rise, as sometimes happened, the results were discarded. The isometric measurements obtained in the manner described do not provide a true measure of the isometric response of the heart but they were found to be a more satisfactory quantitative measure than records of isotonic contractions. Fig 2 shows a series of measurements obtained with the

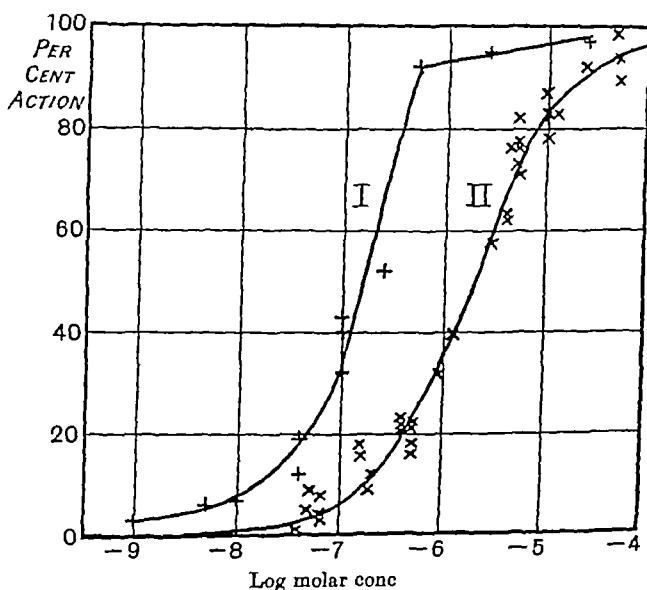


Fig 2 Relation between concentration and action of acetyl choline on the frog's heart. Ordinate percentage of maximal possible action Abscissa logarithm of molecular concentration.

Curve I, isotonic (Straub's cannula), Curve II, isometric (ventricular strip)

whole heart perfused with a Straub's cannula holding 0.5 c.c. of fluid. This preparation was about ten times as sensitive as the isometric strip, but when the concentration of the drug was increased the heart soon reached a condition in which it was unable to lift the apex of the ventricle against the weight of the water, and after this point visible movements continued but no accurate measurement could be obtained.

Heart strips prepared in the manner described and suspended in air will contract regularly and without diminution in force, for at least 30 minutes, and therefore this preparation could be used to measure the absolute quantity of drug needed to produce a particular action, as opposed to the concentration of drug which produced the same action.

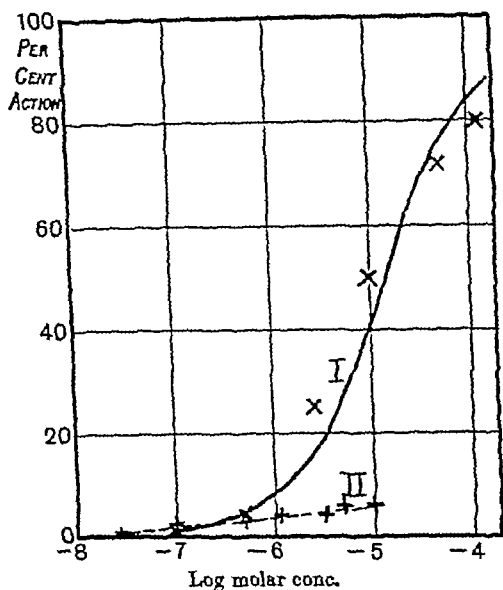


Fig 3. Action of acetyl choline on Rectus abdominis as a function of concentration. I, isotonic, II, isometric. Abscissa and ordinate as in Fig 2

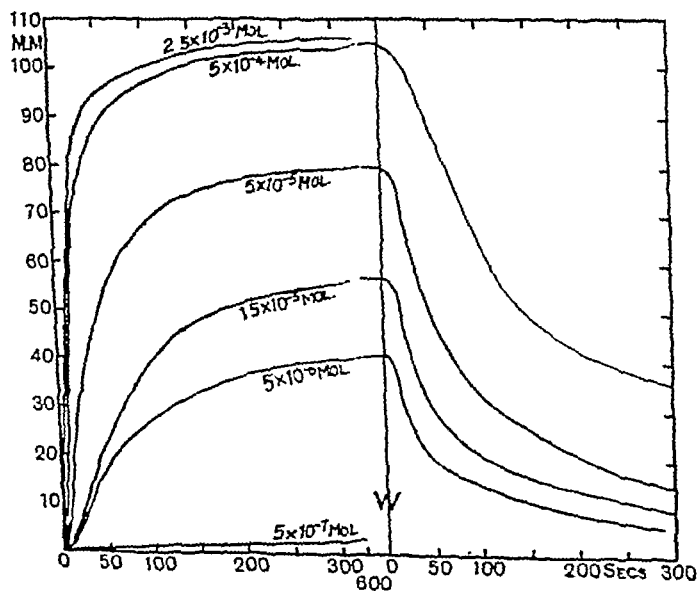


Fig 4 Action of acetyl choline on Rectus abdominis. Specimen curves of isotonic contractions and relaxations. Ordinate—movements of lever point W = wash-out.

when the drug was present in excess. For this purpose a quantity of solution from 0.5 to 2.5 mg. was added to the moist strip. Quantities above 1 mg. were added by means of a capillary pipette while smaller quantities were added by dipping a weighed thread in the solution, weighing the thread again, and then cutting off a length which would contain the correct amount of solution.

Experiments on Rectus abdominis. Acetyl choline produces a contraction of the Rectus abdominis of *R. temporaria*, and acts in concentrations comparable to those which produce depression in the heart.

The isolated Rectus abdominis was suspended in Ringer's fluid and its movements were recorded by an isotonic lever producing a tension on the muscle of about 2 gram. Acetyl choline was found to produce a much more extensive isotonic than isometric response, as is shown in Fig. 3. Tracings of typical contractions produced by acetyl choline are shown in Fig. 4.

The relation between concentration and action. The relation between the concentration of acetyl choline hydrochloride and the isometric response of the frog's ventricle is shown in Fig. 5, and the same relation

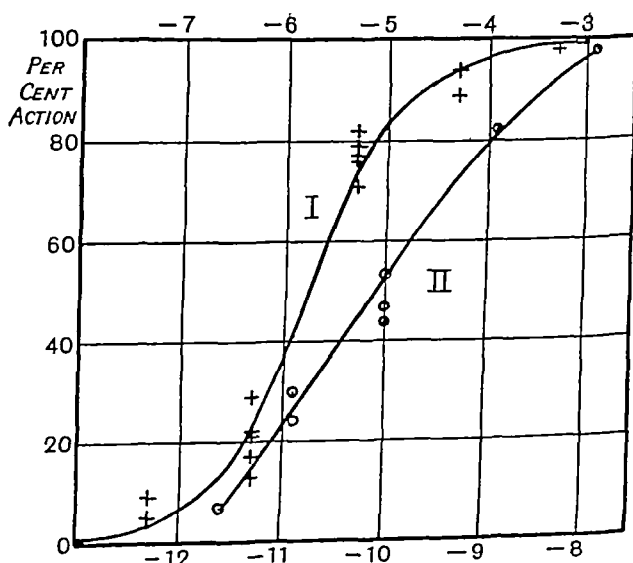


Fig. 5 Action of acetyl choline on heart (isometric) as a function (i) of concentration, and (ii) of absolute amount of drug added. Ordinate action expressed as percentage of maximal possible action. Abscissa along top (and curve I) Log of molar concentration of drug added in excess in dilute solutions. Along bottom (and curve II). Log of gram molecules of drug added in concentrated solution to moist heart.

for the isotonic response of the Rectus abdominis is shown in Fig 6. In both cases the action produced by the drug is expressed as the percentage of the maximal possible action which it can produce. The drug

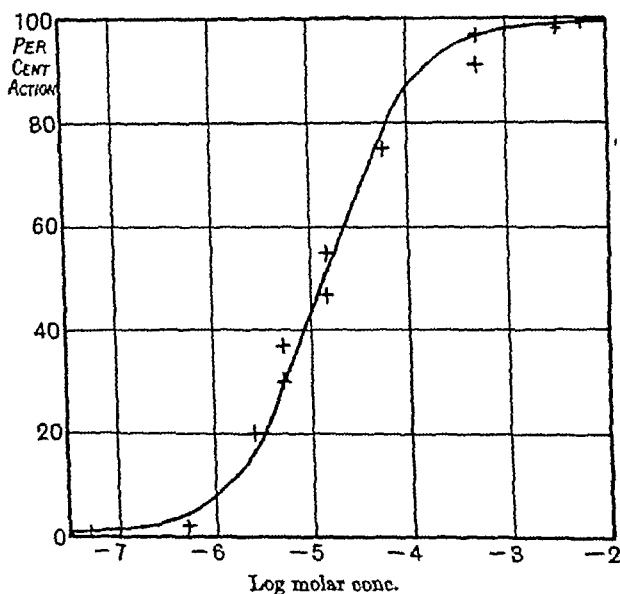


Fig 6 Action of acetyl choline in producing isotonic contraction of the Rectus abdominis as a function of concentration. Ordinate and abscissa as in Fig 2.

produces depression of the heart and the maximal action is total arrest of the heart. By the aid of a reading microscope it is possible to measure contractions which are less than 1 p c of the original amplitude, it is possible, therefore, to measure actions between 90 p c inhibition and complete arrest with considerable accuracy. On the other hand, it is not possible to measure reductions of less than about 5 p c of the normal response. In the case of the Rectus abdominis a contraction of less than 1 mm on the record could be detected, and this was less than 1 p c of a maximal contraction. On the other hand, there is no sure measure of the maximal contraction that the muscle can produce, and therefore the figures for contractions above 90 p c of maximal are somewhat uncertain.

In both cases the figures observed lie along a curve which can be fitted closely by the equation $Kx = \frac{y}{100-y}$, where x = molecular concentration of the drug, y = action produced expressed as percentage of maximal action, K = constant. Curves drawn according to this formula are shown in Fig 2, curve II, Fig 3, curve I, Fig 5, curve I,

and Fig 6 Inspection of these curves shows that the responses observed follow the theoretical curve fairly closely

If $Kx = \frac{y}{100-y}$, x must be a linear function of $\frac{y}{100-y}$ The wide range of concentrations used necessitates the plotting of x on a logarithmic scale and Fig 7 shows $\text{Log } x$ plotted against $\log \frac{y}{100-y}$, and

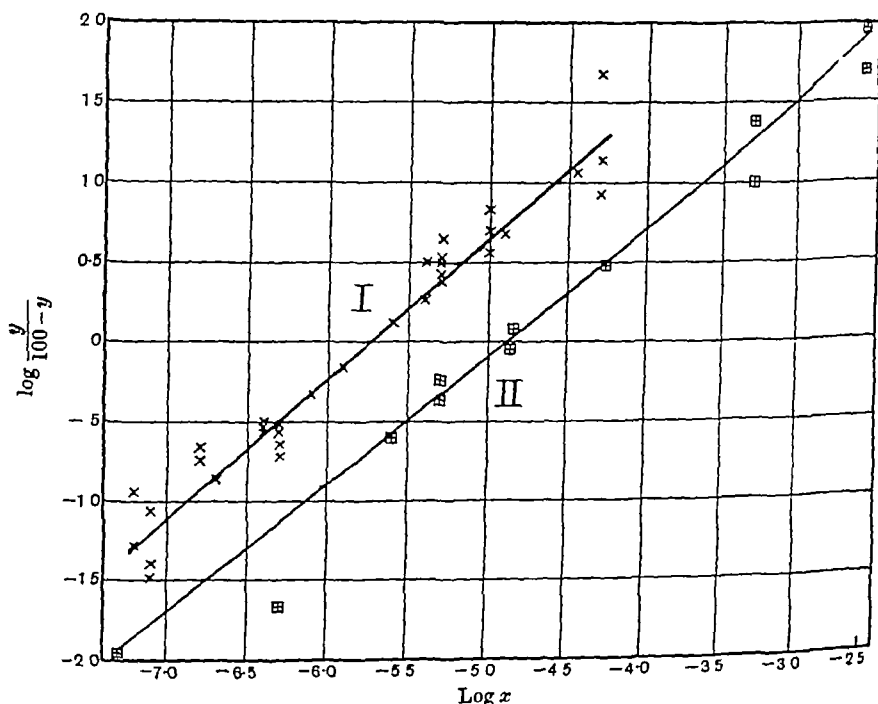


Fig 7 The relation between the concentration of acetyl choline and the response produced.

Ordinate $\text{Log } \frac{y}{100-y}$ Abscissa Log of molar conc. of acetyl choline

Curve I Action on isometric response of frog's ventricle (calculated from exp. in Fig 2, curve II)

Curve II. Action on isotonic contraction of frog's Rectus abdominis (calculated from exp in Fig 6)

inspection shows that the relation is linear Curve I in Fig 7 follows the formula

$$\text{Log } K + \text{Log } x = \text{Log } \frac{y}{100-y}$$

within the limits of error, $\text{Log } K$ being 5.74 Curve II in Fig 7 follows, however, the formula

$$\text{Log } K + 0.8 \text{ Log } x = \text{Log } \frac{y}{100-y}, \text{ or } K x^{0.8} = \frac{y}{100-y}$$

I made a number of experiments to try and determine the value of n in the formula $K x^n = \frac{y}{100-y}$. Unfortunately it is impossible to get very exact quantitative measurements of the responses of either the frog's ventricle or Rectus abdominis. The variations in repeated responses of the tissue to the same concentrations are shown in the figures, and indicate the limits of accuracy of these experiments. I obtained values for n varying from 0.8 to 1.1, and the average value of n was very close to unity. Values for n below one were somewhat more frequent than values above one. I concluded that the probable value of n was one.

The following values were found for K . In 15 experiments upon heart strips contracting isotonically K varied from 33,000,000 to 1,600,000, and the average value was 10,000,000. In 19 experiments upon heart strips, where the response was measured isometrically, K varied from 20,000,000 to 100,000 with an average value of about 1,000,000. In six experiments upon the Rectus abdominis the value of K varied from 400,000 to 10,000 with an average of 40,000. These figures show that one heart may be 200 times more sensitive to acetyl choline than another heart. This is not a seasonal variation, for such differences were observed on successive days.

Graded actions in a single series of experiments could be measured over a 10,000-fold range of concentrations. There was, moreover, a 3000-fold difference between the sensitivity of the most sensitive ventricle and the least sensitive Rectus abdominis. In the case of the ventricle the inhibition of an isometric response was measured and in the case of the Rectus abdominis the production of an isotonic contraction. In all cases the results obtained could be fitted, within the limits of experimental error, by the formula given above. It appears improbable, therefore, that the agreement between the measurements obtained and the formula is a chance one.

The rate of action of acetyl choline. Acetyl choline can act extremely rapidly on the ventricle. Measurements on a rapidly moving drum in one case showed that when the drug was poured directly on to the ventricle a solution of 5×10^{-6} molar acetyl choline produced half its final action in less than a second. The rate of action varied greatly in different preparations, as is shown in Fig. 1.

The action with more dilute solutions was less rapid but in all cases the drug produced half its final action in less than 30 seconds. The rate of removal of the drug on washing out was equally rapid. In this latter case the recovery of the heart was slower after high than after low concentrations of the drug had been applied. In the case of the ventricle

both the rate of action and the rate of removal of acetyl choline appear to be determined chiefly by the rate of diffusion of the drug through the sponge-like tissue of the heart, and hence no satisfactory analysis of these rates can be made

The Rectus abdominis reacts more slowly than the ventricle to acetyl choline, and the rates of reaction and of wash-out are shown in Table I

TABLE I. Rate of action of acetyl choline on Rectus abdominis.

Molecular concentration	5×10^{-8}	5×10^{-7}	5×10^{-6}	1.5×10^{-5}	5×10^{-5}	5×10^{-4}	2.5×10^{-3}	5×10^{-3}
Time in seconds till half final contraction	200	80	64	72	30	14	11	9
Time in seconds after washing out till half relaxation	—	—	22.5	45	67	54	—	—

Unfortunately, the significance of these latter figures is somewhat doubtful, because even to certain types of instantaneous stimulus the Rectus abdominis gives a sluggish response. For example, if the muscle was extended with a weight of 5 grm I found that when the weight was removed the muscle contracted slowly and took nearly three minutes to produce half of the resultant change in length. The rate of change in length, therefore, does not give a measure of the rate of union between the drug and the muscle. Table I shows, however, that in the case of the Rectus abdominis the higher the concentration of the drug the more rapid is its action, and that the time needed for removal of the drug is greater after high than after low concentrations.

The influence of temperature on the action of acetyl choline Alterations in temperature produce no certain effect upon the amount of action produced by acetyl choline upon either the heart or the Rectus abdominis. This is shown in Tables II and III. Increase of temperature, however, increases both the rate of action of the drug and also the rate at which it is washed out, as is shown in Table IV. This table also shows that the rate of contraction, and the rate of relaxation on washing out,

TABLE II. Influence of temperature on the action of acetyl choline on the heart
(The figures show the percentage reduction of the response of the heart)

Mol concentration of acetyl choline hydrochloride	5×10^{-8}	5×10^{-7}	10^{-6}
Temperature	—	32	44
11° C	—	32	44
14° C	9	40	48
20° C	50	30	44
29° C	55	30	37

TABLE III. Influence of temperature on the action of acetyl choline in causing contraction of Rectus abdominis

(The figures show the contraction expressed as a percentage of maximal contraction.)

Mol. concentration of acetyl choline hydrochloride	5×10^{-4}	5×10^{-5}
Temperature		
5° C	41.5	75
15° C	43	77
28° C	29	79

TABLE IV Influence of temperature on the rate of action of acetyl choline on Rectus abdominis

(The figures show the time in seconds until half the action is produced.)

Mol. concentration of acetyl choline hydrochloride	Contraction on introduction of drug		Relaxation on washing out of drug	
	5×10^{-4}	5×10^{-5}	5×10^{-4}	5×10^{-5}
Temperature				
7° C.	108	38	75	85
14° C.	71	34	28	29
27° C.	30	11	25	17

are affected equally by a rise of temperature. This is in accordance with the observation that changes in temperature do not alter the final equilibrium between the drug and the muscle as indicated by the extent of action produced. If we suppose the action of acetyl choline to be proportional to the degree of combination of the drug with some element of the muscle, then the absence of an action of temperature on the final equilibrium state shows that the heat of reaction of the drug with the tissue is comparatively small.

The quantity of drug which reacts with the heart. The results obtained when small amounts of drug are added to the moist ventricular strip show at once that the total quantity of drug necessary to produce an action on the heart is very small. For example, the total amount of drug added which was sufficient to produce a 50 p.c. reduction in the response of the heart strip in Fig. 5 was only 10^{-10} gram molecules.

Figs. 8 and 9 are types of the records from which Fig. 5 was compiled, and they show that the action of acetyl choline on the moist ventricular strip is exactly similar to its action on the strip immersed in solution, and therefore the two sets of actions can be compared quantitatively. Fig. 5 shows, however, that the relation between the quantity of drug added in concentrated solution and the amount of action produced is not the same as that already shown to exist between the concentration of the drug (when present in excess) and its action. For example, the concentration of drug needed to produce 90 p.c. of full inhibition is

both the rate of action and the rate of removal of acetyl choline appear to be determined chiefly by the rate of diffusion of the drug through the sponge-like tissue of the heart, and hence no satisfactory analysis of these rates can be made

The Rectus abdominis reacts more slowly than the ventricle to acetyl choline, and the rates of reaction and of wash-out are shown in Table I

TABLE I Rate of action of acetyl choline on Rectus abdominis.

Molecular concentration	5×10^{-8}	5×10^{-7}	5×10^{-6}	1.5×10^{-5}	5×10^{-5}	5×10^{-4}	2.5×10^{-3}	5×10^{-3}
Time in seconds till half contraction	200	80	64	72	30	14	11	9
Time in seconds after washing out till half relaxation	—	—	22.5	45	67	54	—	—

Unfortunately, the significance of these latter figures is somewhat doubtful, because even to certain types of instantaneous stimulus the Rectus abdominis gives a sluggish response. For example, if the muscle was extended with a weight of 5 grm. I found that when the weight was removed the muscle contracted slowly and took nearly three minutes to produce half of the resultant change in length. The rate of change in length, therefore, does not give a measure of the rate of union between the drug and the muscle. Table I shows, however, that in the case of the Rectus abdominis the higher the concentration of the drug the more rapid is its action, and that the time needed for removal of the drug is greater after high than after low concentrations.

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TABLE II Influence of temperature on the action of acetyl choline on the heart
(The figures show the percentage reduction of the response of the heart.)

Mol. concentration of acetyl choline hydrochloride	5×10^{-6}	5×10^{-7}	10^{-4}
Temperature			
11° C	—	32	44
14° C	9	40	48
20° C	50	30	44
29° C	55	30	37

TABLE III. Influence of temperature on the action of acetyl choline in causing contraction of Rectus abdominis.

(The figures show the contraction expressed as a percentage of maximal contraction.)

Mol. concentration of acetyl choline hydrochloride	5×10^{-4}	5×10^{-3}
Temperature		
5° C.	41.5	75
15° C.	43	77
28° C.	29	79

TABLE IV. Influence of temperature on the rate of action of acetyl choline on Rectus abdominis.

(The figures show the time in seconds until half the action is produced.)

Mol. concentration of acetyl choline hydrochloride	Contraction on introduction of drug		Relaxation on washing out of drug	
	5×10^{-4}	5×10^{-3}	5×10^{-4}	5×10^{-3}
Temperature				
7° C.	108	38	75	85
14° C.	71	34	28	29
27° C.	30	11	25	17

are affected equally by a rise of temperature. This is in accordance with the observation that changes in temperature do not alter the final equilibrium between the drug and the muscle as indicated by the extent of action produced. If we suppose the action of acetyl choline to be proportional to the degree of combination of the drug with some element of the muscle, then the absence of an action of temperature on the final equilibrium state shows that the heat of reaction of the drug with the tissue is comparatively small.

The quantity of drug which reacts with the heart. The results obtained when small amounts of drug are added to the moist ventricular strip show at once that the total quantity of drug necessary to produce an action on the heart is very small. For example, the total amount of drug added which was sufficient to produce a 50 p c reduction in the response of the heart strip in Fig 5 was only 10^{-10} gram molecules.

Figs 8 and 9 are types of the records from which Fig 5 was compiled, and they show that the action of acetyl choline on the moist ventricular strip is exactly similar to its action on the strip immersed in solution, and therefore the two sets of actions can be compared quantitatively. Fig 5 shows however, that the relation between the quantity of drug added in concentrated solution and the amount of action produced is not the same as that already shown to exist between the concentration of the drug (when present in excess) and its action. For example, the concentration of drug needed to produce 90 p c of full inhibition is

100 times that needed to produce 10 p c, but when small quantities of drug are added then the quantity required to produce 90 p c of full inhibition is 1000 times that needed to produce 10 p c

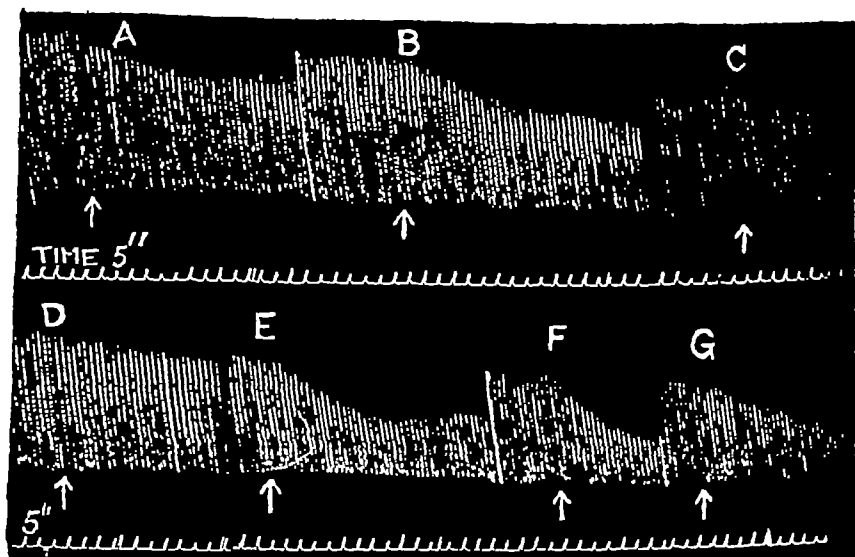


Fig 8 Action of acetyl choline on the isotonic contraction of ventricular strip (weight 30 mg) The curves show either (i) effect of immersion in 5 c.c. solution, or (ii) effect of adding a small quantity of concentrated solution

(i) Molar concentrations of solutions $\times 10^5$ B, 2.5, C, 0.9, F, 5.0 and G, 2.5

(ii) Gram molecules of drug added $\times 10^{12}$ A, 0.6, D, 0.3 and E, 1.5



Fig 9 Action of acetyl choline on isometric response of ventricular strip (weight 25 mg, frequency 20 per min)

Upper curves Effect of immersion in solution. Molecular concentration $\times 10^5$
5, 25, 50, 250, 500, 2500

Lower curves Effect of adding small quantities of drug to moist strip Gram molecules added $\times 10^{12}$ 1.25, 12.5, 125, 1250, 12,500 and 62,500

The quantity of drug entering the muscle cells The figures obtained permit an approximate calculation of the amount of drug actually entering the muscle cells and thus the determination of the relation between this factor and the amount of action produced. In the records shown in Fig 5 the addition of 5 c.c. of solution at a concentration of 4×10^{-6} molar produced a 50 p.c. inhibition of the heart. The 5 c.c. of fluid contained 2×10^{-8} gram mol. of drug, and the action observed could be produced by the addition to the moist strip of 10^{-10} gram mol. Therefore the amount of drug taken up by the heart must have been too small to affect appreciably its concentration in the 5 c.c. of fluid. A 50 p.c. reduction in the response must therefore have been associated with a concentration of 4×10^{-6} molar around the heart cells. When, therefore, 10^{-10} gram mol. added in concentrated solution produce a 50 p.c. reduction in force, sufficient of this quantity must remain in the intercellular fluids of the heart to maintain a concentration of 4×10^{-6} molar and the remainder is presumably absorbed by the heart cells.

I found that moderate pressure between filter paper caused a moist heart strip to lose about 30 p.c. of its weight. A study of sections of the frog's ventricle showed that about 50 p.c. of the area of the section was occupied by intertrabecular spaces. The heart strip may therefore be assumed to consist of about 50 p.c. of muscle cells, and 50 p.c. of intercellular and intertrabecular fluid. With these assumptions the amount of drug that enters the heart cells can be calculated.

TABLE V

The quantity of acetyl choline hydrochloride absorbed by the heart cells.

1	2	3	4	5
Reduction of contraction p.c.	Concentration of dilute solution needed to produce reduction (Mol. conc. $\times 10^7$)	Quantity of drug which produces reduction when added in concentrated solution (G. mols. $\times 10^{-1}$)	Quantity of drug which must remain outside cells to produce the concentration shown in col. 2 (G. mols. $\times 10^{-1}$)	Quantity of drug that can enter cells (G. mols. per mg. of cells $\times 10^{-1}$)
Exp. 31	Ventricular strip 25 mg	Isometric contraction.		
10	2	3.3	2.5	0.06
25	5.6	11	7	0.32
50	18	100	22.5	6.2
75	50	890	63	66
90	200	4000	250	300
95	500	8000	620	590
Exp. 6	Ventricular strip 30 mg	Isotonic contraction		
25	0.13	0.4	0.22	0.012
50	0.35	1.25	0.59	0.044

Table V and Fig 10 give the results of a series of calculations made on these assumptions and they show that the amount of drug taken up by the cells varies approximately as (concentration)². The relation between the amount of action produced (y) and the concentration (x) is however expressed by the formula $Kx = \frac{y}{100-y}$. There is, therefore, no direct relation between the amount of action produced and the amount of drug entering the cells.

In the first experiment in Table V a 10 p c reduction was produced in a strip weighing 25 mg by a 2×10^{-7} mol solution and in the moist strip by a quantity of 3.3×10^{-12} gram mol, this latter quantity would, however, only produce a concentration of 1.3×10^{-7} mol if distributed equally throughout the 25 mg of fluid and tissue. In this case, therefore, the concentration of drug inside the cells must actually have been less than the concentration of drug around the cells. On the other hand, when larger quantities of drug are added its concentration must be considerably greater within than around the cells. These experiments suggest, therefore, that the action of acetyl choline in producing inhibition of the heart cells, and the entrance of the drug into the cells are two independent processes, which have no certain relation to each other.

The amount of drug reaction with a single cell The experiments with small quantities of drug permit the calculation of the amounts of drug that unite with a single cell. Table V shows that a 50 p c reduction in the isometric response of the heart is produced by 6.2×10^{-12} gram mol per milligram of heart cells, and that a demonstrable action occurs when about one-hundredth of this amount enters the heart cells. The isotonic records shown in Fig 8 and Table V show that a demonstrable action is produced when about 10^{-14} gram mol per milligram of heart cells is taken up by the tissue. The average measurements of the spindle shaped heart cells of the frog are $130\mu \times 10\mu$ (Skramlik(3)). Each

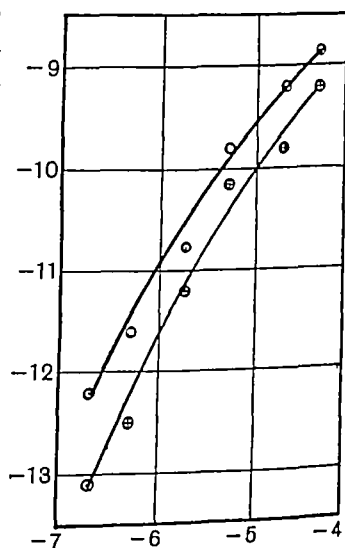


Fig 10 Relation between the concentration of acetyl choline and the amount of drug taken up by the heart cells (two experiments). Ordinate Log of gram molecules of drug taken up by 1 mg of tissue. Abscissa Log of molar concentration of drug.

cell, therefore, has approximately a cubic content of $3400\mu^3$, and a surface area of $2000\mu^2$. One mg of heart tissue will contain, therefore, approximately 300,000 cells, and this will have a total surface of 6 sq cm.

One molecule of a fatty acid in a condensed film on the surface of water covers a surface of about 20×10^{-18} sq cm (Langmuir, Adam), a molecule of cholesterol about 40×10^{-16} sq cm, and each fatty acid chain of lecithin about 50×10^{-16} sq cm (Leathes(4)). It appears probable, therefore, that a molecule of acetyl choline will cover between 20 and 100×10^{-18} sq cm. One grm mol. may be taken as containing 7×10^{23} mol and therefore if 1 mg of heart cells takes up 6.2×10^{-12} grm mol, each heart cell will take up

$$\frac{7 \times 10^{23} \times 6.2 \times 10^{-12}}{3 \times 10^5} = 14,000,000 \text{ mol}$$

and this number will cover an area of the order of 10^{-7} sq cm whereas the cell has an area of 2×10^{-5} sq cm. The smallest quantity of acetyl choline observed to produce an action, namely, 10^{-14} grm mol. per milligram of heart, would provide about 20,000 molecules per cell and these would cover an area of the order of 10^{-10} sq cm.

These figures show that it is impossible that acetyl choline should act by forming a continuous layer over the surface of the heart cells, or by covering any larger area inside the cells.

The amount of drug reacting with the Rectus abdominis A few experiments with small quantities of fluid were performed on the Rectus abdominis. In one case the muscle weighed 0.1 grm and a contraction 50 p.c. of the maximal was produced by immersion in 20 c.c. of 10^{-4} mol. solution, or by adding to the moist muscle 2×10^{-3} grm mol. of acetyl choline. This latter quantity would, however, produce a concentration of 10^{-4} mol. in only 0.02 c.c. This experiment suggests that the concentrated solution diffuses only into about one-fifth of the muscle. The result would be accounted for if the drug diffused into the tissue fluids of the muscle and the amount taken up by the muscle cells was negligible. This accords very well with the results obtained upon the heart, but I am not satisfied that the concentrated solutions diffused evenly throughout the muscle and therefore the experiments cannot be regarded as conclusive.

Discussion These experiments throw some light on the mode of action of acetyl choline on muscle cells. Straub(2) concluded that alkaloids act on heart muscle in two ways, for he showed that some alkaloids such as strychnine and morphine were concentrated in the heart cells whereas other drugs such as muscarine, pilocarpine and adrenaline

were not so concentrated. He concluded that the action of these latter drugs was dependent on the potential difference between their concentrations within and without the cells. This conclusion was based on the fact that muscarine gradually entered the cells and as the drug accumulated in the cells its action on the heart diminished.

This passing off of the action of a drug after prolonged exposure of a tissue to it has also been shown to occur when pilocarpine acts on the rabbit's isolated intestine (Neukirch(5)), and when adrenaline acts on the same tissue (Jendrassik(6)). Wertheimer and Paffrath(7) investigated a series of choline compounds and found that the intensity of their action was inversely proportional to the rate at which they diffused into living tissues. For example, they found that acetyl choline had 1000 times as intense an action as choline upon the isolated gut of the guinea-pig but that choline diffused through frog's skin 1000 times as rapidly as acetyl choline.

My results agree with the above observations in that they show that there is no direct relation between the amount of acetyl choline which enters the heart cells and the action of the drug on the cells. The results described can be explained most readily on the assumption that two processes are occurring, namely, an action of the drug on the cell surface which proceeds very rapidly and which commences at very high dilutions, and secondly, an entry of the drug into the cells which proceeds more slowly and commences at higher concentrations.

I consider it is doubtful whether the partial recovery of the heart on prolonged exposure, as shown in Fig 1, is due to the entry of the drug into the cells, because in the first place this recovery effect is remarkably variable as is shown by the two curves in Fig 1, and secondly, because no similar recovery effect was observed when acetyl choline was allowed to act for periods as long as 2 hours upon the Rectus abdominis. This recovery effect on prolonged exposure of tissues to drugs with reversible actions, although it can be demonstrated with many drugs and various tissues, is certainly not a universal rule. No such recovery is observed when atropine is allowed to act upon the heart, nor when nicotine in low concentrations produces a contraction of the Rectus abdominis. Hill(8) observed that low concentrations of nicotine would maintain a contraction of the Rectus abdominis for an indefinite period, and I found that this was the case for the longest period measured, namely, about 2 hours.

In view of the very great individual variations observed in the sensitivity of hearts to acetyl choline it appears possible that a heart

on prolonged exposure to the drug may acquire some form of tolerance to the drug quite independently of the entry of the drug into the cell

The relation between the concentration and action of acetyl choline in most cases follows the formula $K x = \frac{y}{100-y}$ and the simplest explanation of this fact is to suppose that a reversible monomolecular reaction occurs between the drug and some receptor in the cells. A similar type of curve is observed in the dissociation of dilute solutions of oxyhæmoglobin (Hartridge and Roughton(9)).

My experiments do not exclude the possibility of the formula being $K x^n = \frac{y}{100-y}$ where n is a figure between 0.8 and 1.1. If n is a figure less than 1 the explanation of the relation between concentration and action would be much more difficult.

Various writers have explained the relations observed between the concentration and degree of action of drugs on living tissues on the supposition that the curves are frequency curves due to variations in the susceptibility of the cells on which they act. This type of curve may be anticipated when a drug produces an irreversible action, and Peters(10) showed that the rate of action of mercuric chloride on *Paramæcia* could thus be explained. Shackell(11) has applied this theory to the freely reversible action of adrenaline on arterial rings, and Gaddum(12) has adopted the same explanation for the relation between the concentration of adrenaline and its action on the isolated rabbit's uterus. The figures which he gives can, however, be fitted fairly closely by the formula given above for the action of acetyl choline on the heart. The great range of concentration over which acetyl choline produces a graded response necessitated the plotting of the concentration, in the figures of this paper, on a logarithmic scale, and this renders the relation between concentration and action somewhat less obvious. These curves, as shown in Figs 5 and 6, are rectangular hyperbolæ, and cannot be interpreted as frequency curves. Between the limits of 15 and 85 p.c. of maximal action, however, the relation between log concentration and action is almost linear, and Peters(10) has pointed out that a simple exponential relation of this kind differs very little from the middle portion of a frequency curve. The true relation between concentration and action is therefore only to be established by the observations on the nearly minimal and nearly maximal actions. This fact shows the importance of studying the actions of drugs on tissues under conditions which permit the taking of measurements over the full range of actions of the drug.

CONCLUSIONS

(1) Acetyl choline produces a graded action upon the isolated ventricle and Rectus abdominis of the frog over a 100,000-fold range of concentrations

(2) The relation between the concentration of the drug and the action produced can be expressed by the formula $Kx = \frac{y}{100-y}$, where x = concentration of drug, y = action produced, expressed as maximal possible action, and K = constant

(3) This relation suggests that a reversible monomolecular reaction occurs between the drug and some substance either in the cell or on its surface

(4) The quantity of drug that is fixed by the cells is very small It is calculated that a demonstrable action may be produced on the heart when only 20,000 molecules per cell are fixed these could occupy only a very small fraction of its surface

(5) There does not appear to be any direct relation between the amount of drug entering the cells and the amount of action produced by it These two processes seem therefore to be independent

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THE ANTAGONISM OF ACETYL CHOLINE BY ATROPINE BY A. J. CLARK

(From the Pharmacological Department, University College, London)

THE mode of action of acetyl choline upon the isolated ventricular strip and the Rectus abdominis of the frog has been discussed in a previous paper(1) and the technique used in the experiments described below is the same as there described. The relation between the action (y) expressed as the percentage of maximal possible action, and the concen-

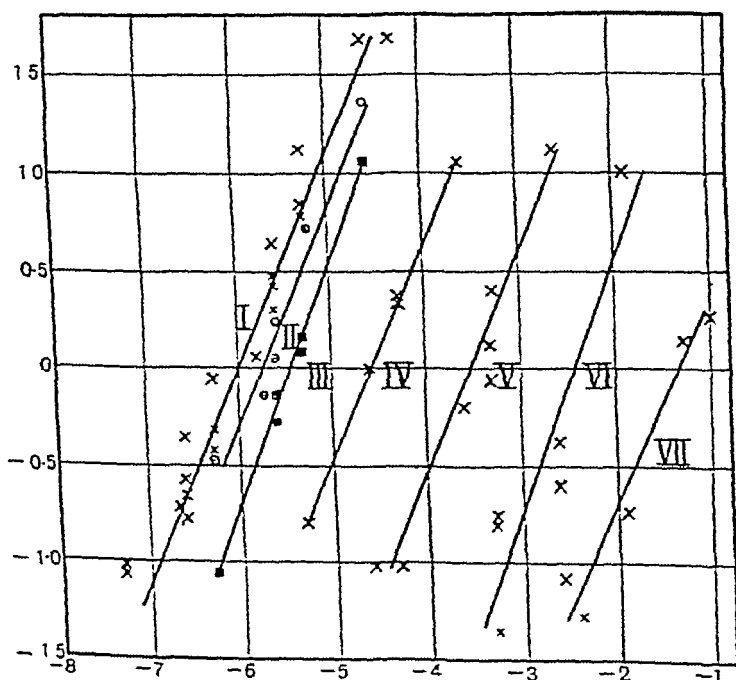


Fig 1 Action of acetyl choline on the heart in presence of atropine x =molar conc. acetyl choline y =percentage reduction in isometric contraction. Ordinate $\text{Log} \frac{y}{100-y}$ abscissa $\text{Log} x$ Molar concentrations of atropine I, no atropine, II, 10^{-5} , III, 10^{-6} , IV, 10^{-7} V 10^{-8} VI, 10^{-9} VII 10^{-10}

tration (x) of acetyl choline has been shown(1) to follow the following formula

$$\text{Formula I } K x = \frac{y}{100-y}$$

The curves I in Figs 1, 4 and 6 show this relation. If the formula is expressed as $K x^n = \frac{y}{100-y}$, then the values for n in these three curves are 1.05, 0.85 and 0.75 respectively, but of these numbers the first is based on many more observations than the other two. As I have been unable to prove that n is for certain either more or less than unity I have assumed that this is its value.

Fig 1 shows the effect on the response of the isolated ventricle to acetyl choline of the presence of increasing concentration of atropine.

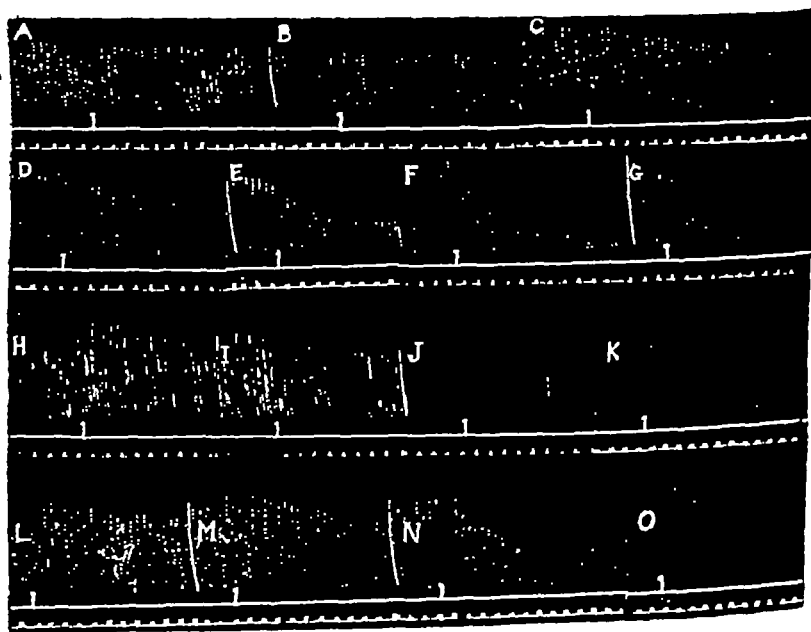


Fig 2 Action of acetyl choline on the frog's ventricle in presence of atropine (Isometric response, stimuli 30 per minute)

A-G, action of acetyl choline in absence of atropine. Molar concentrations A, 5×10^{-6} , B, 10^{-6} , C, 5×10^{-6} D, 10^{-7} , E, 5×10^{-7} F, 5×10^{-6} G, 1.2×10^{-6}

H-K, action of acetyl choline in presence of atropine 3×10^{-6} molar. Molar concentrations Ac. Ch H 10^{-7} I, 2×10^{-7} J, 5×10^{-7} K, 10^{-6}

L-N, atropine 3×10^{-7} molar. Molar concentrations Ac. Ch L, 2.5×10^{-6} , M, 5×10^{-6} , N, 2.5×10^{-6}

O, atropine 10^{-6} molar. Molar concentrations Ac. Ch 2.5×10^{-6}

in the fluid around the tissue, and Fig 2 shows samples of the curves from which Fig 1 was constructed. Fig 3 shows the molar concentrations of atropine and acetyl choline which when present together produce a 50 p c reduction in the response of the heart

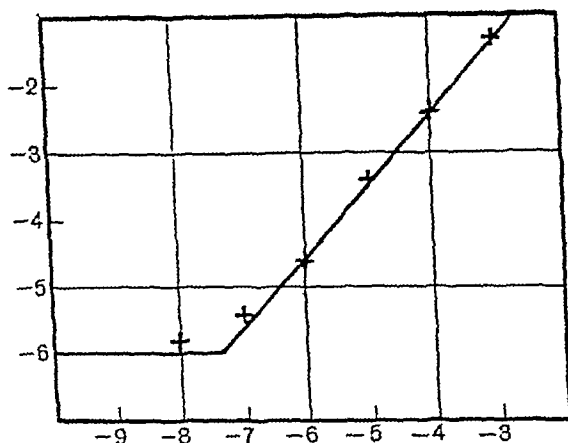


Fig. 3 Concentrations of acetyl choline and atropine which when present together produce 50 p c. reduction in the response of the heart. Ordinate Log of molar conc of acetyl choline. Abscissa Log. of molar conc of atropine

It will be seen that over the greater portion of the curve the relation between the concentrations is

$$\text{Log}(\text{molar conc Acetylcholine}) - \text{Log}(\text{molar conc Atropine}) = \text{constant}$$

Therefore, when constant effects are produced the following relation holds

$$\text{Formula II} \quad \frac{\text{Conc. Ac. Ch.}}{\text{Conc. Atr}} = \text{constant}$$

There is naturally a threshold for the concentration of acetyl choline, namely the concentration needed to produce 50 p c reduction in the absence of atropine (10^{-6} molar). The values obtained suggest that there is also a threshold value for the concentration of atropine between 10^{-9} and 10^{-8} molar, below which it produces no demonstrable effect. The figures for the action of acetyl choline when the concentration of atropine is 10^{-7} molar or less show a deviation from formula II. This slight deviation was also observed in other experiments, and I believe that it cannot be explained as due to experimental errors. I have been unable to devise any simple formula which will cover this deviation, but if the figures for these lowest active concentrations of atropine be ignored, then formula II holds.

A combination of formulæ I and II gives the following

$$\text{Formula III} \quad K \frac{\text{Conc Ac Ch}}{\text{Conc Atr}} = \frac{y}{100-y}$$

This formula is the same as that found by Hufner(2) to express the combination of hæmoglobin with oxygen in the presence of carbon monoxide, a conclusion confirmed later by Douglas, Haldane and Haldane(3)

Figures calculated by means of formula III agree very well with the results shown in Fig 1, in this experiment $K = 0.028$ Table I

TABLE I Concentration of atropine constant at 10^{-5} molar

Molar conc acetylcholine $\times 10^5$		3.2	10	32	100	320
Percentage of action produced by acetyl choline (Complete arrest=100)	{ Calculated	8	22	47	73.5	90
	{ Observed	8	20	42	76	90

shows the calculated and observed results when the concentration of atropine is taken as constant (10^{-5} molar) Table II shows the calculated

TABLE II. Concentration of acetyl choline constant at 3.2×10^{-3} molar

Molar conc. atropine $\times 10^5$		1000	100	10	1
Percentage of action produced by acetyl choline (Complete arrest=100)	{ Calculated	8	47	90	99
	{ Observed	6	34	94	—

and observed results when the concentration of acetyl choline is taken as constant at 3.2×10^{-3} molar Fig 3 shows the observed results when the action produced is taken as constant

In these experiments the concentrations of acetyl choline used ranged from 0.1 to 10^{-8} molar and the concentrations of atropine from 10^{-3} to 10^{-7} molar and the agreement between the observed and calculated figures over this very wide range of concentrations must be considered satisfactory

The values obtained for K in experiments on different hearts varied. The figures obtained in four experiments were as follows 0.5, 0.2, 0.2, 0.028. This variation is probably connected with the great differences observed in the susceptibilities of different hearts to acetyl choline

The experiments show that in any single heart an equal effect is produced as the resultant of the action of the two drugs as long as $\frac{\text{Conc. Ac Ch}}{\text{Conc. Atr}}$ remains constant, provided that the concentration of atropine is sufficient to produce a well-marked effect. This relation is in accordance with that found by Cushny(4) for the antagonism by atropine of the action of pilocarpine on the salivary gland of the dog. He found that one part of atropine antagonised about eight parts of

pilocarpine, and that this ratio remained constant even when the doses were varied fifty-fold.

Figures given by Broom and Clark(5) show a similar relationship for the antagonism of adrenaline by ergotamine in the isolated rabbit's uterus. These figures show that approximately equal effects are produced with the following concentrations of the two drugs

Mol. conc. ergotamine $\times 10^3$	0	4	8	16	32	53
Mol. conc. adrenaline $\times 10^3$	22	112	220	220-440	440-880	880

The ratio between the concentrations of ergotamine and adrenaline is about 1 to 25 and remains nearly constant.

Gaddum(6) gives results that show that the ratio between the concentrations of adrenaline and ergotamine that produce equal actions on the isolated rabbit's uterus remains approximately constant. The rabbit's isolated uterus is not well adapted to accurate quantitative work, but these results show that the antagonism between adrenaline and ergotamine follows a course very similar to the antagonism between atropine and acetyl choline.

The results obtained with the Rectus abdominis muscle are not quite

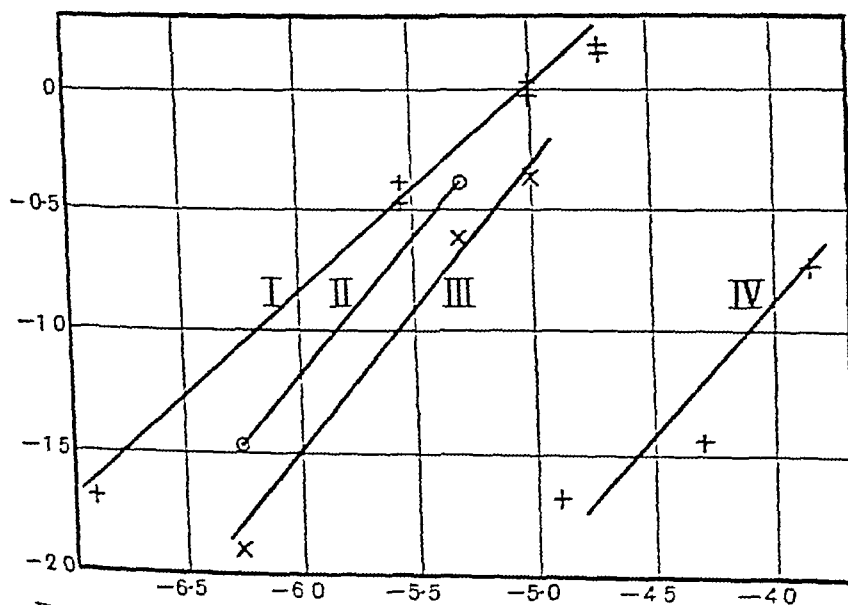


Fig. 4. Action of acetyl choline on the Pectus abdominis, in presence of atropine. x = molar conc. acetyl choline. y = contraction produced expressed as percentage of maximal contraction. Ordinate and abscissa as in Fig. 1. Molar concentrations of atropine: I, no atropine, II, 3.3×10^{-3} , III, 10^{-4} , IV, 10^{-3} .

so simple Fig 4 shows a sample of the measurements obtained and Fig 5 the concentrations of acetyl choline and atropine which, when acting together, produce a contraction amounting to 20 p c of the maximal These results follow the formula

Formula IV

$$K \frac{\text{Conc Ac. Ch.}}{(\text{Conc Atr})^{1/2}} = \frac{y}{100 - y}$$

This formula gives satisfactory results over the range of concentrations at which atropine produces a well-marked effect

The following values were found for K with the Rectus abdominis 0.32, 0.25, 0.16, 0.1 A relation of a somewhat similar type was found by Le Heux, Storm van Leeuwen and van den Broeke(7), who studied the action of atropine and pilocarpine on the isolated gut of the rabbit They showed that Lidth de Jeude(8) was mistaken

in supposing that the action of atropine was not dependent on the concentration of the drug, and that the relation between atropine and pilocarpine could be expressed by the statement that equal effects were produced when

$$\text{Conc Atr} = K (\text{Conc Pilo})^n$$

In one of their experiments n was 1.5

The Quantity of Atropine Uniting with the Heart I have described in the previous paper(1) a method of estimating the amount of drug actually reacting with tissues by adding to the moist ventricular strip a minute quantity of fluid This method was used with atropine The reaction of a heart strip to solutions of acetyl choline was first determined, and then the reaction of the preparation to minute quantities of acetyl choline After these figures had been determined a small quantity of atropine was added to the moist strip in 1 mg of fluid After 10 minutes the reaction of the strip to a minute quantity of acetyl choline was measured The strip was then thoroughly washed and the process recommenced

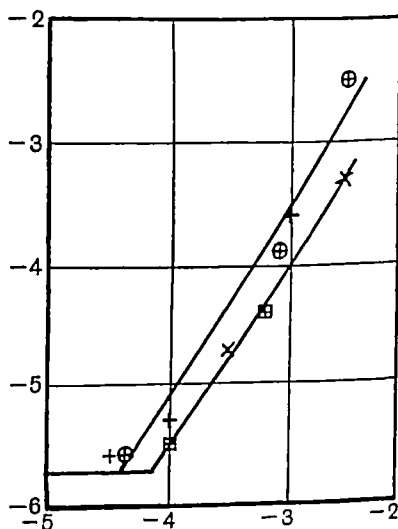


Fig 5 Concentrations of acetyl choline and atropine which together produce 20 p c of maximal contraction in the Rectus abdominis Ordinate and abscissa as in Fig 3 (The curve is constructed from four different experiments)

The results obtained are shown in Fig 6 The addition of 10^{-10} gm mol of atropine reduces tenfold the sensitivity of the strip to acetyl choline Accurate measurements are very difficult to obtain with this

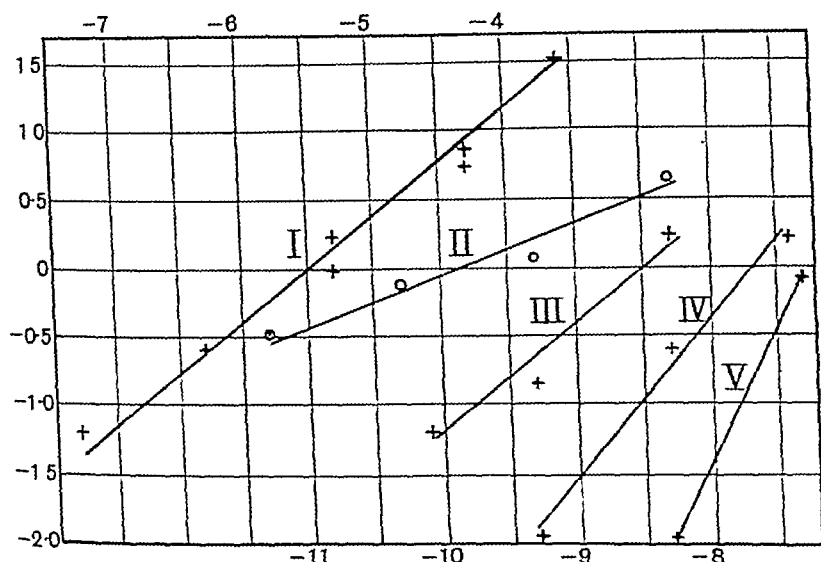


Fig 6 Antagonism of small absolute quantities of acetyl choline by small absolute quantities of atropine in a ventricular strip (weight 15 mg) Ordinate $\text{Log} \frac{y}{100-y}$ (y = percentage reduction in response) Abscissa along top—Log of molar conc. acetyl choline (curve I), along bottom—Log of gram mols of acetyl choline added (curves II-V)

I. Response of heart immersed in 5 c.c. of solution (no atropine)

II-V Response of moist ventricular strip to small quantities of acetyl choline

Gram mol. of atropine present II, Nil III, 10^{-10} IV, 10^{-9} V, 10^{-8}

method, and therefore I cannot say definitely whether, when equal effects are produced, the fraction $\frac{\text{Grm Mols Atr}}{\text{Grm Mols Ac Ch}}$ remains constant or not, Fig 7 suggests that the ratio probably does remain constant

These experiments are quoted merely to show that the amount of atropine which actually unites with the heart tissues is extremely small, and of the same order as the amount of acetyl choline

The Rate of Action of Atropine When atropine is introduced to a heart depressed by the presence of acetyl choline, the length of time taken by the atropine to produce half its action in antagonising the acetyl choline varies from 20 to 60 seconds The rate of action of atropine is less than half the rate of action of acetyl choline Rates of action as rapid as these cannot, however, be determined with any accuracy because of the error

due to the delay caused by drug diffusing into the sponge-like tissue of the ventricle

The rate of wash-out of atropine is very much slower than its rate of action. The rate of wash-out can be calculated by testing the heart repeatedly with acetyl choline after removal of the atropine solution. In this way the concentration of atropine can be calculated that would be needed to produce the observed interference with the action of acetyl choline. Table III shows figures thus obtained, and indicates that about half the drug is removed in about 10 minutes.

Acetyl choline is washed out of the heart in about half a minute and therefore the rate of release of atropine is far slower than that of acetyl choline.

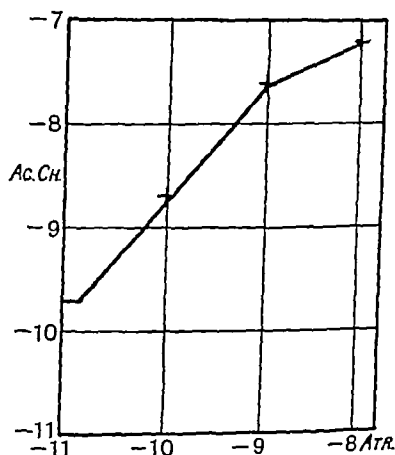


Fig 7 Antagonism of small absolute quantities of acetyl choline by small absolute quantities of atropine. Ordinate Log of gram mols of acetyl choline needed to produce a 50 p.c. reduction in the response. Abscissa Log of gram mols of atropine present.

TABLE III Effects produced on heart by acetyl choline $10^{-3.6}$ molar during the wash out of 10^{-5} molar atropine. The above concentration of acetyl choline produced 99.5 p.c. reduction of response in the heart before the introduction of atropine.

Time in minutes since atropine removed	0	2	5	10	20	40	60	90	120
Percentage reduction in force of response	20	28	37	44	62.5	70	75	84	87
Molar conc atropine which would diminish the action of $10^{-3.6}$ molar Ac. Ch. to this response	10^{-3}	$10^{-3.1}$	$10^{-3.2}$	$10^{-3.35}$	$10^{-3.7}$	$10^{-3.9}$	10^{-4}	$10^{-4.15}$	10^{-4}

The Mode of Antagonism of Atropine and Acetyl Choline The figures in Table III show at once that the antagonism between these two drugs depends upon the atropine being fixed in some manner by the tissues for the atropine continues to exert its antagonistic action on acetyl choline for a long time after thorough and repeated washing away of all the drug around the heart cells. This excludes the possibility of the antagonism being due to any reaction between the drugs outside the heart cells.

The two drugs do not appear to react when the atropine is fixed on

the heart cells, for the application of concentrated solutions of acetyl choline does not hasten the rate at which the atropine is washed out.

Straub(9) suggested that atropine rendered tissues impermeable to drugs like acetyl choline. He concluded that in the heart of *Aplysia muscarina* acted only when entering or leaving the tissues, but he admitted that this was not the case in the frog's heart. There is, indeed, no evidence that the action of acetyl choline depends upon the drug entering the heart cells. I showed in a previous paper(1) that the drug does enter, but that there was no clear relation between the amount entering the heart cells and the amount of action which it produced. The relation found to hold over a wide range of concentrations for the antagonism between acetyl choline and atropine, namely

$$K \frac{\text{Conc. Ac. Ch.}}{\text{Conc. Atr}} = \frac{y}{100 - y}$$

is the same as that found for the antagonism of oxygen and carbon monoxide when the two substances react with hæmoglobin. There is, however, a fundamental difference between these two processes because oxygen and carbon monoxide displace each other from combination with hæmoglobin. This does not appear to be the case with acetyl choline and atropine, for when a heart which is recovering from atropine is exposed to a concentration of acetyl choline sufficient to produce arrest, this does not increase the rate of recovery of the heart from the atropine. Acetyl choline in excess, therefore, does not react with the atropine to neutralise it, nor does the former drug displace the latter from the heart cells.

Atropine and acetyl choline, therefore, appear to be attached to different receptors in the heart cells and their antagonism appears to be an antagonism of effects rather than of combination. The evidence available is insufficient to make the publication of further speculation as to the nature of the antagonism profitable.

CONCLUSIONS

(1) The action of acetyl choline and atropine on the heart, when both are present, can be expressed by the formula

$$K \frac{\text{Conc. Ac. Ch.}}{\text{Conc. Atr}} = \frac{y}{100 - y}$$

(y = action produced by acetyl choline expressed as percentage of maximal possible action, and K is a constant). This formula only holds when atropine is present in a concentration sufficient to produce a well-marked action.

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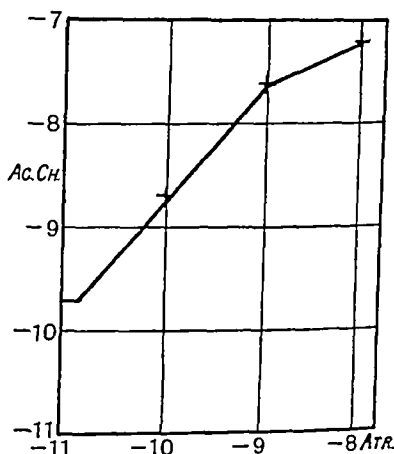


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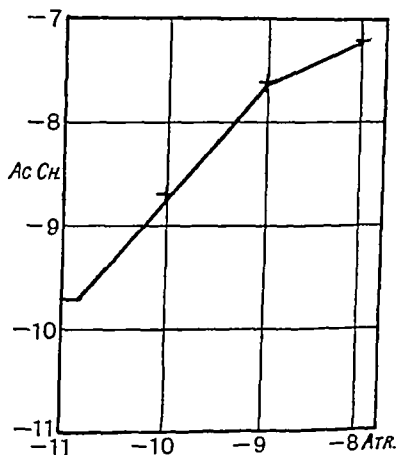


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(2) The action of the two drugs on the Rectus abdominis can be expressed by the formula

$$K \frac{\text{Conc Ac Ch}}{(\text{Conc Atr})^{1/2}} = \frac{y}{100 - y}$$

(3) The amount of atropine that unites with the heart muscle is very small. A quantity of 1.4×10^{-11} gram mols per milligram of heart cells suffices to increase tenfold the quantity of acetyl choline needed to produce a given percentage of reduction in the response of the heart.

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(2) The action of the two drugs on the Rectus abdominis can be expressed by the formula

$$K \frac{\text{Conc Ac Ch}}{(\text{Conc Atr})^{1/2}} = \frac{y}{100-y}$$

(3) The amount of atropine that unites with the heart muscle is very small. A quantity of 1.4×10^{-11} gram mols per milligram of heart cells suffices to increase tenfold the quantity of acetyl choline needed to produce a given percentage of reduction in the response of the heart.

The expenses of this research were covered by a grant from the Government Grants Committee of the Royal Society.

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EFFECT OF PARATHYROID FEEDING ON THE THYROID By DOROTHY WOODMAN

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School of Medicine for Women)*

Two hypotheses have been put forward to explain the relationship of the thyroid and parathyroid. The first one suggests that the two glands are so related in function that a deficiency of the one is compensated for by hypertrophy of the other. The older experiments of Vincent and Jolly(1), Halpenny and Thompson(2) supported this view, but Vincent and Arnason(3) were unable to confirm their results.

Scott Williamson(4) believes the thyroids and parathyroids are related anatomically, physiologically and pathologically. He suggests that a fluid matter (not colloid) is found in the sinusoids of the thyroid during secretory activity and can be traced to the tubes of the parathyroid.

The second hypothesis suggests that though the two glands are related anatomically they are antagonistic in their physiological function. This is well supported by a comparison of their effects on calcium metabolism.

The chief differences between the two glands have been summarised by Cowdrey(5) by a comparison of the effects produced by atrophy, removal or administration of extracts of the two glands.

The following experiments were carried out to show the effect of parathyroid feeding on the structure of the thyroid, and hence obtain information with regard to the functional relationship of the two glands.

Very few experiments correlating the respective functions and structure of the thyroid and parathyroid have been carried out. Kojima(6) fed rats with parathyroid but his results are not satisfactory as the rats had previously been fed with thyroid. Halpenny and Thompson(2) give diagrams which indicate that after parathyroidectomy the vesicles of the thyroid are larger, more irregular, and appear more active.

Methods

Adult and young rats of both sexes were used for these experiments.

Feeding The actual method of feeding and the diet given is reported by Woodman(7) Doses of 0.02 grm. desiccated parathyroid were given daily for periods varying with the different series from 2-5 weeks. The rats were weighed twice a week.

Histology The rats were killed by chloroform and a small portion of the trachea, to which the thyroid and parathyroids were attached, removed, fixed in formal-acetic-Muller and prepared for embedding in the usual way. The appearance, especially the colour, of the thyroid was noticed on removal.

The slides were examined to determine the degree of activity of the thyroid at death, the degree of activity being judged by the following points:

(a) The amount of colloid—and its appearance, i.e. degree of staining, and presence or absence of vacuoles.

(b) Character of vesicular epithelium—whether flattened, cubical, or columnar.

(c) Size, number and irregularity in shape of vesicles.

Points (a) and (b) were specially observed, since several observers have put forward the view that the amount of stored colloid (and therefore the type of vesicular epithelium) varies inversely with the degree of activity of the gland (Courrier(8), Scott Williamson(4)).

Parathyroid The parathyroid was examined to determine if any structural change had occurred, special attention being paid to the fact that wide variations in types of cells and amounts of fibrous tissue are normally found.

Results

The effects of the parathyroid feeding on the structure of the thyroid were so consistent that only a summary is given.

These results were obtained from (a) six litters of young rats including 48 rats of both sexes, (b) 29 adult rats of both sexes.

Control Thyroid Typical appearance. Fair amount of colloid present, vacuolated in many cases. Epithelium cubical or columnar. Gland appeared resting or active and in most cases very vascular (see Fig. 1).

Parathyroid Vascular in many cases. Variation in amounts of fibrous tissue present was found.

Parathyroid-fed rats *Thyroid* Appeared inactive in every case. Inactivity most marked in rats fed with parathyroid for longer period. Vesicles distended with colloid and in some cases so distended as to give an appearance similar to "colloid goitre" (see Fig. 2).



Fig 1 Thyroid (control) Note small vesicles
Columnar epithelium. Vacuolated colloid



Fig 2 Thyroid (parathyroid fed animal) Note larger vesicles
distended with colloid. Epithelium flattened

Parathyroid Adult rats showed more fibrous tissue than young rats, but on the whole the amount of fibrous tissue present lay within the limits of the normal variations

Discussion of Results

Parathyroids The parathyroids of controls are generally formed of masses of cells, and only traces of fibrous tissue are found. On the other hand, the parathyroids of experimental rats appeared in most cases to consist of cells more loosely arranged and separated by islands or strands of fibrous tissue. Hoskins(9) and McCarrison(10) have both pointed out that the parathyroid may either consist of compact masses of cells and no strands of fibrous tissue, or the cells may be more loosely packed and separated by well-marked reticular tissue. McCarrison states that both types may even be seen in the same glandule. Since these variations in structure may be found in the normal gland, the apparent increase of fibrous tissue in several of the parathyroid-fed rats cannot be ascribed to a specific effect of the parathyroid feeding.

Thyroid Macroscopically, the thyroids of rats which had received parathyroid were a pale pink in colour as compared with the healthy red colour of controls. This change in colour of the thyroid was also found by Cameron and Carmichael(11) after thyroid feeding, which, as is discussed below, produced the same histological effect as reported here after parathyroid feeding. Histologically, the appearance of the thyroid in the rats which had received parathyroid was quite different from that in the controls. The vesicles were large, distended with colloid, and vesicular epithelium was flattened. The effect was more marked where animals received parathyroid for a longer time.

The assumption is, that parathyroid feeding, since it produces an accumulation of colloid in the gland, has inhibited the activity of the thyroid. These results might be interpreted in two ways, either in support of the suggestion that the thyroid and parathyroid are compensatory to a certain extent, or that they are antagonistic in function.

Cameron and Carmichael(11) and Kojima(6) reported that thyroid feeding produced an inactive appearance of the thyroid. The results of parathyroid feeding appear to produce an identical change in the thyroid and therefore suggest, at first sight, that the two glands have a similar function and are able to compensate for each other. But, if the effect of parathyroid feeding is to replace the function of the thyroid in the body thereby causing a storage of its colloid, then one would expect similar changes in body weight and growth to those produced by

thyroid feeding It has already been reported by Woodman(7), that no marked effect on growth was found after parathyroid feeding, and certainly not the decrease in weight which is found with thyroid feeding Thus it is questionable whether the inhibition of the thyroid is due to a replacement of its function by the parathyroid

On the other hand, the influence of the thyroid and parathyroid on both calcium and carbohydrate metabolism has suggested that they are antagonistic Kojima(6) found a storage of colloid in the thyroid after parathyroid feeding but did not continue to investigate whether the effect was due to the parathyroid or to previous thyroid feeding The results of the experiments reported here suggest that his results were due to the parathyroid feeding Critchley(12) reported that 11 cases of Graves' disease were treated with parathyroid, and in every case nervousness and tachycardia were diminished, and in five cases the goitre was reduced The results of the experiments reported here taken in conjunction with the above report of Critchley and the results of Halpenny and Thompson(2) suggest that the parathyroid normally inhibits the thyroid

SUMMARY AND CONCLUSIONS

(1) Administration of parathyroid produces no marked structural change in the parathyroid

(2) The thyroid of rats fed on parathyroid presents an inactive appearance as shown by the increased amount of colloid present and the distension of the vesicles

(3) It is suggested that the effect of parathyroid feeding on the thyroid is due to the antagonism of the two glands rather than to a replacement of the thyroid function by the administered parathyroid

In conclusion I wish to thank Prof Cullis for her help and criticism throughout this work, and also the Royal Society for a grant allotted towards the expenses incurred

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STUDIES ON THE INTERNAL SECRETIONS OF THE OVARY I

The distribution in the ovary of the œstrus-producing hormone BY A. S. PARKES (*Beit Memorial*

Research Fellow) AND C. W. BELLERBY

(*From the Department of Physiology and Biochemistry,
University College, London*)

1 INTRODUCTION

By experimental excision it has been shown quite definitely that the presence of the ovary is essential for

- (a) The development of the accessory organs of reproduction
- (b) The occurrence of the cyclic changes which constitute the œstrous cycle

(c) The persistence of pregnancy (during the early stages) and the development of the mammary glands

Further, since transplantation of the ovary has not the same inhibitory effect as excision, it must be supposed that the means of control is hormonal. In addition, functional correlation between the ovaries and the accessory organs suggests that the maturation of the Graafian follicles results in œstrus, and that the subsequently developed corpora lutea are responsible for the maintenance of pregnancy, the development of the mammary glands, and for the inhibition of œstrus during pregnancy. Since the accessory organs develop in the absence of both mature follicles and corpora lutea, neither of these can be necessarily associated with their development.

Of the three possible tests for internal secretion the third and most fundamental, extraction of the active principle from the organ in question, has only been satisfactorily applied to one of these functions of the ovary, i.e. the production of œstrus. Ovarian extracts definitely capable of causing development of the accessory organs after early ovariectomy or capable of taking the place of the corpus luteum have not yet been prepared. Whether or not three separate and distinct internal secretions of the ovary exist, it is certain that the œstrus-producing hormone does not represent the entire internal secretory apparatus of the ovary.

Extraction of ovaries with the idea of obtaining substances capable of taking the place of the ovaries in ovariectomised animals was originally begun many years ago, but the early work was severely handicapped in many ways

The first real advance was made by Adler⁽¹⁾, who stated that he could produce, by injection of aqueous extracts of whole ovaries into virgin animals, changes in the genital organs, especially the uterus, which resembled the appearance of natural rut. His results were checked by histological examination

Iscovesco⁽¹⁶⁾ was the first to employ organic solvents to extract the active substance. He obtained from the whole ovary a substance which produced on injection, congestion, hæmorrhage, and augmentation of weight and volume of the uterus of normal adult animals. These uteri were two or three times the weight and volume of those of the control animals. Fellner⁽¹⁰⁾ shortly afterwards described the preparation of a substance by alcoholic extraction of whole ovaries, which, on injection into ovariectomised rabbits, produced symptoms of œstrus such as hyperplasia of the uterus and vagina. Okinschitz⁽¹⁸⁾ prepared extracts from whole ovaries, liquor folliculi and corpora lutea, by grinding with saline and glycerol. He claimed that subcutaneous injection of the first two extracts retarded the rate of atrophy of the uterus of ovariectomised rabbits but that the last-named extract produced no such effect. Seitz, Wintz and Fingerhut⁽²⁰⁾ in the same year prepared from the corpus luteum by extraction with alcohol and acetone, two substances, one of which promoted menstruation, whilst the other had an inhibitory action. Later, in 1916, they stated that an active substance could be obtained from whole ovaries and placenta. Herrmann and Fränkel⁽¹⁵⁾ claimed to have separated from whole ovaries and placenta a lipid substance, which when injected into immature rabbits, caused development of the whole genitalia, namely, the vulva, vagina, uterus and ovaries. From 1915 onwards Frank and his co-workers^(11, 12, 13) published a series of papers, in which their observations agreed with those of Herrmann and Fränkel. They also obtained an active substance from corpora lutea. Their extracts were tested on immature rabbits and on castrated rats. Aschner⁽⁴⁾ showed that subcutaneous injection of extracts of ovary and placenta produced symptoms of œstrus in castrated guinea-pigs

In spite, however, of this large amount of work, it was not until 1923, when Allen and Doisy⁽³⁾ published a series of papers, that the accurate testing of extracts was recorded. These authors point out the

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In spite, however, of this large amount of work, it was not until 1923, when Allen and Doisy(3) published a series of papers, that the accurate testing of extracts was recorded. These authors point out the

fundamental weakness of the greater part of this earlier work, and emphasise two points of major importance

(a) That no substance can be said to be capable of taking the place of the ovary unless tested on ovariectomised animals. Most of the earlier workers tested extracts on "virgin" animals

(b) That the criteria of oestrus which were used by earlier workers had little or no meaning

Allen and Doisy's contention that these facts practically invalidate the whole of the earlier work is clearly justified, and in any case there is no necessity to give more than the above brief account of the work performed before that of Allen and Doisy

The comparatively recent discovery of Long and Evans⁽¹⁷⁾ for rats, and Allen⁽²⁾ for mice, that the various stages of the oestrous cycle could be followed accurately by examination of the changes in the vaginal contents, and that these changes in the vagina ceased after ovariectomy has provided an easy and accurate method of testing the oestrus-producing activity of ovarian extracts, and the vaginal smear technique has really made possible the recent development of ovarian extraction work

Allen and Doisy, the first authors to apply the vaginal examination technique to the testing of extracts, as a result of a prolonged and carefully controlled investigation, came to important conclusions which may be briefly summarised here

(a) An oestrus-producing extract can be made from liquor folliculi, and to a lesser degree, from residual tissue. The occurrence of the hormone in the residual tissue is, however, put down by Allen and Doisy to the incomplete removal of liquor folliculi

(b) No oestrus-producing extract can be made from the corpora lutea

(c) As a result of their work the authors conclude that the oestrus producing hormone is elaborated by the maturing follicle

Since the publication of the Allen and Doisy papers, many workers have confirmed and amplified their results. Courrier^(5, 6), using guinea-pigs as test animals, worked with liquor folliculi only, and observed oestrous phenomena in ovariectomised guinea-pigs after injection. When injected during pregnancy, the active principle appeared to pass across the placenta and cause hyperæmia of the uterus of the female fetuses. Dickens, Dodds and Wright⁽⁸⁾ "were unable to secure the large yields from liquor folliculi, and from human placenta, described by these workers (Allen and Doisy). The yields obtained by us from

liquor folliculi and from the residual ovarian tissue are of the same order. Our findings, therefore, do not support the contention that the active principle is confined to the liquor folliculi." Their comparison of yields from residual ovarian tissue (after removal of follicles and corpora lutea) and liquor folliculi is, however, based on one extraction only, so it must be admitted that the whole tendency of the work relating to the extraction of an oestrus-producing substance from the ovary has so far been to emphasise the importance of the part played by the Graafian follicles in producing oestrus.

Recently, however, one of us (Parkes(19)) has shown, by means of destroying the follicles with X-rays, that a perfectly normal oestrous and uterine cycle may occur quite independently of the presence of any follicles in the ovary. Since the irradiated ovaries contained no cyclic structures whatever, and consisted mainly of tissue which appears to be hypertrophied germinal epithelium, the experiments reveal the ovary in quite a different light, and the specific object of this present paper is to consider the distribution of the hormone between the follicles and residual tissue in order to ascertain whether the work on ovarian extraction is really as incompatible as it appears to be with the fact that an oestrous cycle occurs in the absence of Graafian follicles.

2 METHODS AND MATERIALS

Material. Cow ovaries have furnished the chief material for the work described in this paper. Cow ovaries, as compared with pig, have the disadvantage of having only one or two large follicles at most, and therefore of providing less liquor folliculi, but they have the advantage of having a more compact stroma, and of having corpora lutea which are much more easily removed than in the case of the pig. Further, it is rarely possible to obtain pig ovaries with really large follicles, owing to the fact that pigs in oestrus are seldom killed in this country. Some few batches of pig material have, however, been used, and in addition one lot of definitely anoestrous sheep ovaries and one sample of horse ovaries have been extracted. As samples of immature ovaries three samples from young porkers and one sample from young heifers have been obtained. From this material extracts have been made of

- (1) Whole ovaries, without corpora lutea
- (2) Liquor folliculi
- (3) Young or mature solid corpora lutea
- (4) Hollow corpora lutea
- (5) Residual tissue

Method of extraction The ovaries were kept in cold storage at -15° until sent to the laboratory and they were thus still frozen when received. As the solidified follicular liquid could be cleanly "shelled out" without difficulty, this greatly facilitated clean dissection in cases where extraction of separate parts was desired. The ovaries were then slit open and any other follicles and corpora lutea dissected out. The corpora lutea and residual tissues were thoroughly washed in two or three changes of water to free them from possible contamination with the liquor folliculi. The method of extraction of the material was the same in all cases, and consisted of a modification of the first process described by Herrmann and Fränkel. The preliminary stage of treatment of the liquor folliculi was different and will be discussed later.

After being weighed, the tissue was finely minced, mixed with twice its weight of alcohol and allowed to stand for about 24 hours. The alcoholic solution was then decanted off, and the tissue pressed out and freed from any liquid. This material was then extracted for 6-9 hours under a reflux condenser, twice its wet weight of alcohol being again used. The alcoholic extracts were then combined, filtered through flannel and evaporated to a small volume *in vacuo* until free from alcohol. The thick suspension was shaken up with about twice its volume of ether. The emulsion which formed separated into two layers on standing for about 1 hour. The aqueous layer was drawn off, and shaken up with a fresh quantity of ether. It was found by experience that two extractions were as a rule quite sufficient. The ethereal solutions were combined, and evaporated to a small volume. Acetone was then added, and the precipitate of lipoids was filtered off through a hard filter paper. The clear yellow filtrate was then evaporated to dryness, and the oily brown residue rubbed up with a small quantity of absolute alcohol, the clear liquid decanted off, filtered, and then placed in the cold store for 24 hours. The clear solution was again filtered off from the precipitate of fats and cholesterol and evaporated to dryness. No attempt at further purification was made of this residue. As was mentioned above, the preliminary treatment of the liquor folliculi varied from this procedure. Two volumes of cold alcohol were added to one volume of the liquor and the thick white mixture was then boiled under a reflux condenser for about 1 hour. This treatment caused flocculation of the proteins, which could then be easily filtered off by means of flannel. The solid material was re-extracted with the same volume of boiling alcohol for 6-9 hours, the alcoholic filtrates combined and taken through the process as detailed above. This process was found to be necessary, as we have found that even after precipitation of the liquor folliculi with hot alcohol the finely divided precipitate that formed not only took some days to settle, but was also practically unfilterable. We found also that the precipitate after settling apparently carried down most of the active substance with it.

As a control 3 kilos of lean beef were worked up by the above process. No positive results were obtained.

Testing The oily residue obtained at the end of the process described above was emulsified in 1 p.c. Na_2CO_3 , a suitable concentration for injection being about 1 grm. in 50 c.c. Ovariectomised mice were used as test animals, and in this connection two points of interest were observed. In the first place Davenport(7) has shown that a considerable percentage of ovariectomised mice may regenerate ovarian tissue within a comparatively short time of the operation, and his results have been amply confirmed on our own material. Thus the possibility is always present that an apparently positive result may be due to regenerated

ovary This does not seem to have been appreciated by previous workers To overcome this factor as far as possible, routine testing animals were only used for a short time after ovariectomy, and an interval of greater length than the normal oestrous cycle was allowed between successive tests This latter precaution in more than one case revealed spontaneous oestrous activity on the part of the ovariectomised animal The percentage of regeneration was, however, kept below that found by Davenport by removing the whole ovarian region, including capsule and fat body, at the time of operation In the second place we have confirmed the fact observed by Dickens, Dodds and Wright that the amount of active extract required to produce oestrous phenomena increases with increasing interval after ovariectomy To secure comparable results this again means that animals should not be used for any great time after ovariectomy

Vaginal smears were taken from the test animals at intervals of 24 hours during the whole time they were being used, and to test an extract two injections of the Na_2CO_3 emulsion were given at an interval of 12-18 hours Where the extract was active, pro-oestrous changes in the vaginal smear (for account of these, see Allen(2) and Parkes(19)), occurred within 24 hours of the last injection The ease and completeness with which the injections were absorbed seemed to depend primarily on the amount of cholesterol present, excessive amounts leading, as noted by Dodds, to granulations To arrive at the minimum active dose of any given extract a trial injection was first given This amount was then increased or decreased according to whether it was inactive or active until the least amount necessary to produce oestrous symptoms was arrived at From this the total number of "mouse units" (M U) in the extract was calculated, and the activity of the original tissue or fluid was calculated in mouse units per kilogram (M U K) In addition, the activity of the crude yield is calculated as mouse units per gram (M U G), and the amount of crude yield as grams per kilogram of original tissue (G K)

3 EXTRACTS

Whole ovaries The data to be considered here relate to one batch of mature pig ovaries and one batch of mature cow ovaries, both containing follicles in all stages of development but no corpora lutea, three batches of immature pig ovaries, two batches of immature cow ovaries and one batch of anæstrous sheep ovaries, all of which contained only very small follicles (1-3 mm) The following table summarises this material

TABLE I Yields from whole ovaries

Material	Amount (gram)	Yield (gram)	G K	M U	M U G	M. U R	Notes
Mature pig	1140	3 17	2 82	250	78 9	219	—
Mature cow	1980	3 75	1 89	580	154 6	293	—
Immature cow	110	15	5 31	8	53 3	73	—
Immature cow	180	85	4 72	63	74 2	350	—
Immature pig	30	08	6 25	5	62 5	166	Only 10 pairs of ovaries
Immature pig	280	1 65	5 90	62	37 6	222	—
Immature pig	460	2 27	4 81	125	56 8	273	—
Anæstrous sheep	270	1 34	4 84	55	41 1	203	—

Liquor folliculi Extracts of liquor folliculi from 11 different samples of ovaries are available for discussion. Of these ovaries three batches are from pigs, one lot from horses, and seven from cows. In very few cases were follicles of any size obtained from the pig and the cow material probably provided a greater percentage of ovaries approaching œstrus than did the pig. The horse ovaries, taken from animals sent to slaughter were in all probability in the anæstrous stage. The following table summarises the liquor folliculi extractions.

TABLE II. Yields from liquor folliculi

Material	Amount (c c)	Yield	G K	M U	M U G	M. U R
Cow	105	06	57	5	83 4	48
"	565	27	48	21	77 8	37
"	650	49	75	60	122 5	92
"	520	62	1 19	51	82 4	98
"	85	19	2 23	67	352 7	788
"	320	31	97	33	106 5	103
"	255	35	1 40	134	382 9	520
Pig	153	13	85	6	46 1	39
"	300	10	34	7	70 0	23
"	280	37	1 31	21	56 8	76
Horse	150	16	1 07	17	106 2	113
Total	3383	3 05	9	422	138 4	125

Residual tissue Nine estimations of the activity of ovarian tissue after the removal of the follicles and corpora lutea have been made. Of these eight relate to residues of batches of ovaries obtained for liquor folliculi and the remaining one to residue from ovaries selected for obtaining corpora lutea. The residual tissue extractions are summarised in Table III.

Corpora lutea Although 11 batches of solid mature corpora lutea have so far been extracted by the same method no œstrus active extract has so far been produced. It has, however, been found to be possible to extract the œstrus-producing extract from young fluid containing corpora lutea of the cow. This work is being published.

TABLE III. Yields from residual tissue

Material	Amount	Yield	G. E.	M. U.	M. U. G.	M. U. K.	Notes
Cow	1030	4.15	4.01	245	59.0	238	—
"	390	1.20	3.08	124	103.3	326	—
"	490	2.23	4.51	167	74.9	341	—
"	360	.92	2.55	63	68.5	175	—
"	410	1.12	2.71	125	111.6	310	—
Horse	1140	2.27	1.99	31	13.6	27	—
Pig	260	2.07	7.98	59	28.5	227	—
"	140	1.27	9.09	121	95.3	865	—
Cow	560	1.65	2.95	84	50.9	150	Corpora lutea taken from all ovaries
Total	4780	16.88	3.51	1019	60.5	213	—

separately, but it may be pointed out here that since in most cases hollow corpora lutea cannot be distinguished from others except by cutting them, the contamination of the mature corpora lutea with the young bodies may have given rise to the positive results reported for corpora lutea in general by various authors

4 DISCUSSION

Analysis of the results given above shows that our general results are in agreement with those of Allen and Doisy, and of Dodds as regards the order of amounts of the oestrus-producing extract obtainable from ovaries, but it is significant that in common with Dodds we have failed to obtain the large yields from liquor folliculi which are reported by Allen and Doisy. In order to check the completeness of our extractions the extracted residues and the discarded fractions from all the experiments were preserved. All this material, including aqueous suspensions and lipid precipitates, corresponding to some 10 kg of fresh ovary, has been re-extracted, and the total yield from this source has been negligible. This shows that our extractions have been practically as complete as possible by present methods.

The main points brought out by our data are discussed below.

Yield of crude extract. The following table summarises the results as regards the yield of crude extract per kilogram of original material.

TABLE IV. Yields of crude extract

Material	Amount (gm.)	Crude extract (gm.)	Yield per kg (gm.)
Mature whole ovaries	3.120	6.92	2.22
Immature whole ovaries	1.330	6.34	4.76
Liquor folliculi	3.383	3.05	0.9
Residual tissue	4.780	16.88	3.5
Total	12.613	33.19	2.6

The follicle fluid, therefore, gives the lowest yield of crude extract, but as pointed out below, this extract is purer than the others. The residual tissue and immature ovaries give the highest crude yield, and the whole mature ovaries, containing large follicles and tissue naturally give a yield midway between that of residual tissue and follicles. The largest yields obtained were ones of 80 and 91 gm per kg from pig residual tissue.

Mouse units per kilogram (M U K) The two yields for mature whole ovaries were 219 M U K and 293 M U K, while liquor folliculi yields ranged between 23 and 788. The latter variation, though great, is quite comparable with the yields found by Dodds, Dickens and Wright. The residual yields ranged from 27 to 865 M U K, the whole of the residual tissue extracted averaging 213 M U K.

Calculating ovaries as a whole, by far the lowest M U K was obtained from the horse. The total horse material used was 1290 gm, and only 44 M U were obtained from this, giving a M U K of 35. This low yield is probably accounted for by the fact that the ovaries in question were obtained from horses out of the breeding season.

Specific variation in active yield The following table compares the M U K obtained from the total pig material and the total cow material, including mature ovaries only.

TABLE V Comparison of yields from cow and pig ovaries

Material	Follicles			Residual tissue			Total M U K
	Total amount	M U	M U K	Total amount	M U	M U K	
Cow	2500	371	148	3240	808	219	205
Pig	733	34	47	400	180	450	103

The obvious conclusion from this table is that in the total amounts of residue and liquor folliculi extracted there appears to be but little difference between the cow and the pig material. It is, however, remarkable that the pig liquor compares so poorly with that from the cow, whereas the pig residue is almost double the cow residue. The significance of this, if any, is not clear.

Comparison between activity of liquor folliculi and residual tissue Eight comparisons of the M U K of liquor folliculi with the corresponding residual tissue of the same ovaries are available.

As regards the M U per kilo of original tissue Table VI shows that in five out of the eight cases the residual tissue has the greater activity, the follicle being most active in three only of the series. On the total

TABLE VI. Comparison between liquor and residual tissue

Material	M U G		M U K.	
	Laq fol	Corresponding residue	Laq fol	Corresponding residue
Horse	106.2	13.6	113	27
Pig	70.0	28.5	23	227
"	56.8	95.3	75	865
Cow	122.5	59.0	92	238
"	82.4	103.3	98	326
"	352.7	74.9	788	341
"	106.5	68.5	103	175
"	382.9	111.6	526	310
Total	150.6	61.4	152	207

figures for the whole series, the activity of the residual tissue is some 50 p c higher than that of the liquor folliculi. As regards the M U per gram of extract the follicle material has a greater activity in six out of the eight cases and on the total figures the activity is two and a half times as great as that of the residual tissue. This means either that the crude extract from liquor contains less inactive matter or else that the inactive matter in follicular extract is easier to remove by freezing methods than is the inactive matter in residual tissue extracts.

From the above table, taken in conjunction with Tables II and III, a curious correlation is obtained. For the eight batches of ovaries dealt with in Table V, batches which were separated into liquor folliculi and residual tissue, it is clearly possible to obtain a rough guide to the average stage of development of the follicle, that is to say, their average size, by considering the amount of liquor obtained relative to the amount of residual tissue of the same batch, *i.e.* by considering the ratio weight of residual tissue/vol of follicle fluid. At the same time it is possible to find the relative weight-for-weight activity of the residual tissue and liquor folliculi of each batch by considering the ratio M U K of follicle fluid/M U K of residual tissue. The values for these ratios are given

TABLE VII. Relative activity of residual tissue and liquor folliculi indices and size of follicle indices.

Material	Amount of tissue		M U K.		Wt. residual/ Vol. follicle	M U K. follicle/ M U K. residual
	Residual (gm.)	Follicle (c c)	Follicle	Residual		
Horse	1140	150	113	27	7.6	4.2
Cow	490	85	788	341	5.8	2.3
"	410	255	526	310	1.6	1.7
"	1030	650	92	238	1.6	39
"	360	320	103	175	1.1	59
Pig	260	300	23	227	87	11
Cow	390	520	98	326	75	30
Pig	140	280	75	865	50	68

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TABLE VII. Relative activity of residual tissue and liquor folliculi indices and size of follicle indices.

Maternal	Amount of tissue		M.U.K.		Wt. residual/ Vol. follicle	M.U.K. follicle/ M.U.K. residual
	Residual (gram.)	Follicle (c.c.)	Follicle	Residual		
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"	360	320	103	175	1.1	59
Pig	260	300	23	227	87	11
Cow	390	520	98	326	75	30
Pig	140	280	75	865	50	08

above in Table VII, in which the batches are arranged in descending order of magnitude of weight of residual tissue/vol of liquor folliculi

The two sets of indices are shown in the following diagram

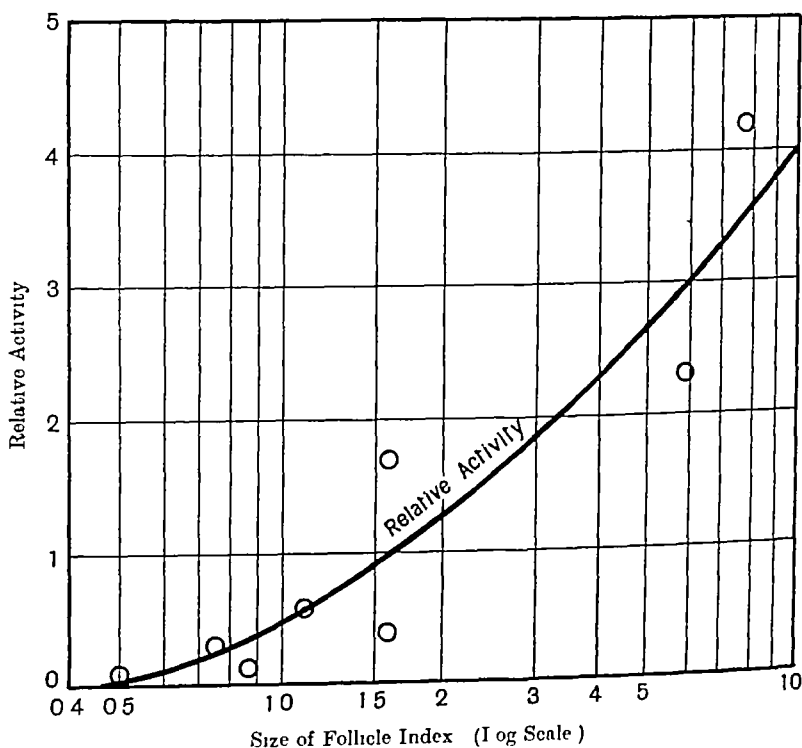


Fig 1

So far as this material goes, it would certainly appear, therefore, that the smaller the bulk of the follicle compared with that of the residual tissue, *i.e.* the more immature the follicles, the greater weight-for-weight oestrus-producing activity they exhibit compared with the residual. Put another way, it may be said that the greater the development of the follicles, the relatively more active the residual tissue. The actual meaning of this is obscure, but it is all against the view that the hormone is elaborated by the maturing follicle.

In an attempt to press further the absence of necessary connection between the oestrus hormone and maturing follicles, a number of samples of immature ovaries and one sample of anoestrous sheep material were obtained. The immature ovaries were carefully selected as being

definitely pre-puberty organs, and contained only very small follicles. Since sheep only appear on œstrus during July—Sept, ovaries obtained, as in this case, during the late autumn, are definitely from the anœstrous period. Neither large follicles nor corpora lutea were present in any of those used. The yields obtained from these various ovaries without mature or even semi-mature follicles are shown in Table I. The two samples of immature cow ovaries gave yields of 73 μ U K and 350 μ U K, while the three samples of immature pig ovaries yielded 166, 222 and 273 μ U K. The anœstrous sheep ovaries gave a yield of 203 μ U K. None of these yields is strikingly low, and the general order of yield is quite comparable with those found for adult ovaries.

In addition to these data one extraction was made from residual tissue of a batch of ovaries specially selected for containing large corpora lutea. None of these ovaries contained follicles of appreciable size, but the yield, shown in the bottom line but one of Table III, from the residual tissue, was 150 μ U K.

Site of origin of the œstrus-producing hormone. The significance of this observed distribution lies in its bearing upon the question of the site of origin of the œstrus hormone. The whole tendency of previous extraction work has been to emphasise the importance of the follicle in governing œstrus, Allen and Doisy go so far as to claim that the hormone is elaborated by the follicle itself, but the results described here show that if the possibility of extracting the substance from a certain part of the ovary is to be considered evidence that it is produced there, then the stroma tissue of the ovary has at least as good a claim to be considered the site of origin as have the follicles. Evidence drawn from extraction values cannot therefore be considered to be of any assistance in determining the site of origin of the œstrus-producing hormone and as a result of the X-ray work mentioned above (Parkes(19)), it is possible to say quite definitely that under certain conditions at least, ovaries consisting entirely of extra-follicular tissue can produce a perfectly normal œstrous cycle.

In view of this conclusion, we suggest that "folliculin," the only term which so far appears to have been given to the œstrus-producing principle, is quite misleading, and we suggest that "œstrin" or some such term, showing immediately its physiological significance, should be applied to the œstrus-producing principle of the ovary.

The regulation of the œstrous cycle. The results recorded above, taken in conjunction with the facts reported by other workers, make it clear that œstrin can be produced from ovaries during any state of the

œstrous cycle, whether the ovaries contain large or small follicles, corpora lutea of ovulation or even corpora lutea of pregnancy. How then is nymphomania avoided, and how is the periodic occurrence of œstrus regulated? The alternation of œstrus and growing corpus luteum in the normal œstrous cycle suggests that the corpora lutea have an inhibitory effect and that œstrus is allowed to appear by the cessation of the corpora lutea function, but, though persistent corpora lutea undoubtedly have an inhibitory action, such an explanation does not cover the whole of the phenomena to be explained. In the case of most of the irradiated ovaries (Parkes(19)), for instance, no luteal tissue was present, but the normal cyclic phenomena in the accessory organs were still observed. The most probable explanation is that some "threshold" concentration of the hormone has to be obtained before œstrous changes can be produced, and that the presence of persistent corpora lutea delays the attainment of this threshold. Such a hypothesis is, however, purely speculative at the moment, and much more work will be needed before the actual means of the regulation of the œstrous cycle can be ascertained.

5 SUMMARY

(1) Œstrus-producing extracts of ovaries have been obtained of about the same order of activity as recorded by Allen and Doisy, and by Dodds, Dickens and Wright.

(2) The methods used, a modification of the first process described by Herrmann and Fränkel, gave yields of crude extract varying between 0.9 and 4.76 grm per kilo, the lower figure being for liquor folliculi.

(3) The mouse units (minimum amount to produce œstrus in ovariectomised mouse) obtained per kilogram of original tissue varied enormously, but averaged between 200 and 300.

(4) Out of eight cases, the residual tissue had a greater activity than the corresponding liquor folliculi in five, while in the remaining three cases the liquor folliculi had the greater weight for weight activity.

(5) From this material there appears to be some correlation between the size of the follicles, and the relative activity of liquor folliculi and residual tissue.

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THE INFLUENCE OF THE VAGUS ON THE ISLETS
OF LANGERHANS Part II The effect of cutting the
vagus upon sugar tolerance By G A CLARK

(From the Physiological Laboratory, Sheffield University)

IN Part I of this paper⁽¹⁾ experimental evidence was given, which supported the view of de Corral⁽²⁾ and of Macleod and his co-workers⁽³⁾, that the right vagus contains secretory fibres to the islets of Langerhans Britton⁽⁴⁾ has recently confirmed the earlier observations, using methods which eliminate sources of error that were possible in the experiments of de Corral and Macleod The significance of these secretory fibres, however, remains obscure

Since, for many months after section of the right vagus in the neck, rabbits appear perfectly healthy, gain in weight and show no obvious change in blood-sugar level, it is clear that the essential function of the islets in the metabolism of sugar is carried on in spite of the nerves having been cut The production of insulin must continue, and the islets themselves apparently respond to variations in the composition of the blood with suitably varying activity, this local adjustment being sufficient at any rate for the needs of animals leading a placid life in confinement In other circumstances the rich nerve supply which de Castro⁽⁵⁾, Gentes⁽⁶⁾ and Pensa⁽⁷⁾ have traced from the right vagus to the islets may intervene When, for instance, a relatively large amount of glucose is rapidly introduced into the circulation, it seems possible that a reaction might be set up somewhere that leads to such intervention

The methods employed and precautions adopted in the present investigation are similar to those previously described⁽¹⁾ When the vagus was cut in the neck, the section was always made below the origin of the cardiac branches, and a piece of the nerve removed to prevent regeneration, which might otherwise have occurred in those experiments of long duration If secretory nerves to the islets play a part in the normal secretion of insulin, a decreased sugar tolerance would be expected after section of those nerves, for it is evident that an increased supply of insulin is necessary to deal with the hyperglycæmia resulting from the intravenous injection of glucose Tests, in which 1 grm of pure glucose per kilo of body weight was injected intravenously in 10 p.c solution

into rabbits, gave no indication of decreased tolerance following right vagotomy within a period of about two months. On the contrary, in a few cases an increase in tolerance was suggested, and in order to investigate this, a more severe test was imposed. The animal was given four intravenous injections of 1 grm of glucose per kilo at two-hourly intervals and the blood-sugar estimated every half-hour after the last injection. In the normal rabbit thus treated the blood-sugar does not return to the fasting level for 3 hours or more (Table I and Fig 1), but about 14 days after section of the right vagus the fasting level is reached within 1 or $1\frac{1}{2}$ hours (Table I and Fig 3). In Table I the initial blood-sugar levels in all cases before vagotomy appear high, because, at the time of observation, the hyperglycæmia due to the third injection had not passed off. After vagotomy, the normal fasting level had already been reached when the fourth injection was given, two hours after the third. The fasting sugar level is given in a separate column. No tolerance test was done earlier than 10 days after operation.

TABLE I

Rabbit	Hours after last injection	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	Fasting B. S.
1	Before vagotomy	140	244	183	144	138	133	131	—	105
	After "	102	195	122	112	102	113	—	—	
2	Before "	130	224	202	175	165	145	137	130	115
	After "	121	206	117	120	108	123	—	—	
3	Before "	130	308	261	210	177	150	136	—	118
	After "	120	230	130	123	124	120	—	—	
4	Before "	138	221	181	160	152	148	134	—	112
	After "	113	229	100	121	104	113	116	—	
5	Before "	131	212	184	171	150	138	125	122	112
	After "	—	—	—	—	—	—	—	—	
6	Before "	—	—	—	—	—	—	—	—	117
	After "	114	219	106	112	094	100	115	106	
Av	Before "	134	242	202	172	156	143	133	(126)	—
	After "	114	216	115	112	106	114	(116)	(106)	

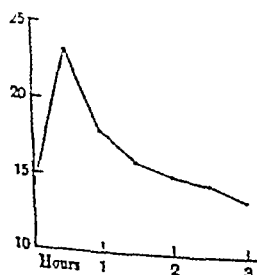


Fig 1

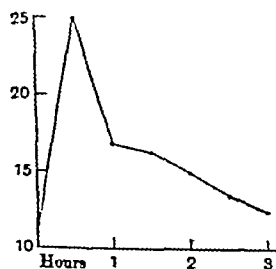


Fig 2.

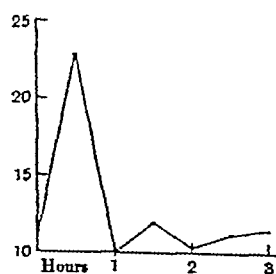


Fig 3.

A striking condition of increased tolerance is thus found in every one of the five rabbits on which the test was successfully carried out. The comparison of the average values at the different times before and after vagotomy shows this clearly. The result in each case conforms to these averages.

This condition, however, was found to pass gradually off, and after about two months the hyperglycemia following a single injection of glucose (1 gm. per kilo) began to last longer than is the case in normal animals, that is to say, the increased tolerance regularly found during the first weeks after vagotomy is succeeded by diminished tolerance. This is still, in the four rabbits that have been studied longest, evident after 21, 25, 33 and 55 weeks respectively, this second phase of the changes induced by vagotomy, a condition of diminished tolerance, seems, therefore, to be permanent.

Table II gives the response of one of these rabbits to 1 gm. of glucose per kilo at different intervals after operation and the figures are typical of those found in the other cases. It will be noticed that there is a tendency for the fasting sugar level to rise to a maximum at about the fourth month after vagotomy, thereafter returning to a lower level. In the last two tests given it will be seen that the return of the curve to the normal level is interrupted by a secondary rise, this was also seen in the other cases.

TABLE II

Hours after glucose	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3
Before vagotomy	116	212	130	100	106	118	—
	120	245	132	106	115	120	—
After							
5 weeks	115	208	116	103	120	116	—
11 "	123	240	159	136	130	124	—
16 "	126	252	150	138	135	130	—
20 "	121	236	177	135	122	124	—
31 "	114	262	145	149	130	115	—
41 "	106	232	145	150	127	117	—
55 "	105	213	130	137	140	135	115

These two stages in the tolerance changes are, therefore, definite and distinct. It is evident that section of secretory nerves to the islets of Langerhans will not explain the first of them, the increased tolerance during the first weeks after the operation. The section must interfere with other influences affecting the sugar in the blood.

Some of the ways in which the phenomenon might be accounted for have been examined.

1. As the recurrent laryngeal fibres were in all cases severed in the vagus trunk, it was necessary to eliminate any possible effect of this

section on the thyroid gland bearing on the result. In three rabbits, therefore, the right recurrent laryngeal nerve alone was cut after the normal response to a fourth glucose injection had been determined as described above. Two weeks later the response to the test was unaltered (Table III and Fig 2), but two weeks after subsequent division of the right vagus trunk two animals showed the typical increased tolerance (Table III and Fig 3). Rabbit 9 died under the anæsthetic during section of the vagus.

TABLE III.

Rabbit	Hours after last injection	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3
3	{ Normal	130	308	261	210	177	150	136
	{ Sect. rt. rec. laryng n.	142	243	173	160	152	142	133
	{ " " vagus	120	230	130	123	124	120	—
4	{ Normal	138	221	181	160	152	148	134
	{ Sect. rt. rec. laryng n.	130	250	168	163	150	135	125
	{ " " vagus	113	229	100	121	104	113	116
9	{ Normal	130	225	162	152	144	140	132
	{ Sect. rt. rec. laryng n.	136	234	190	154	166	173	162

2 The increased tolerance following section of the right vagus might be due to a diminution of the normal rate at which sugar leaves the liver, this hypothesis involving in turn the supposition that in the right vagus are contained, either fibres to the liver that directly stimulate the breakdown of glycogen into sugar, or, alternatively, fibres to the suprarenal which by increasing the discharge of adrenaline would produce the same effect indirectly. It seemed that it might be possible to test this conjecture by the reaction to insulin. If cutting the right vagus arrests the normal delivery of sugar into the blood-stream by the liver, the fall in blood sugar, brought about in the reaction to insulin injected after this operation, might be more rapid than in the normal animal. Two experiments were, therefore, done in which the blood-sugar was estimated every half-hour after 1 c.c. insulin (B.D.H.), diluted 1 in 10, was given intravenously before, and about 14 days after section of the right vagus. It will be seen from Table IV that in neither case was a greater degree of

TABLE IV

	Hours after insulin	0	$\frac{1}{2}$	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$
Rabbit 10	Normal	{ 108	—	081	—	073	082	090
		{ 104	—	081	—	070	076	090
	After vagotomy	{ 105	—	083	—	095	098	101
		{ 106	093	082	—	092	099	102
Rabbit 11	Normal	{ 093	—	066	—	058	067	080
		{ 093	—	062	—	059	070	082
	After vagotomy	{ 095	—	061	—	060	074	080
		{ 095	—	064	062	064	077	090

hypoglycæmia observed after vagotomy All the tests were made under identical conditions, and each was repeated at an interval of 2 or 3 days

3 Or, thirdly, since it appears that in some conditions, the placid life of a hutch rabbit for instance, no gross disturbance of the regulation of the sugar content of the blood is to be detected, after cutting the right vagus, and the islets must, therefore, be still functioning and acted on by variations in the composition of the blood, it is not impossible that as the acini of the pancreas are stimulated to secretion by something brought to them in the blood, and, nevertheless, receive a supply from the vagus of augmentor and also inhibitory nerves, so, too, the islets may receive inhibitory as well as augmentor fibres from the vagus If this were so, the fact that departures from the normal level of blood-sugar are almost always, if not always, in the direction of hyperglycæmia would indicate that inhibition of the islets was the rule and augmentation the exception Cutting the vagus in that case might increase the reaction of the islets to a sudden rise of blood-sugar, and in this way the increased tolerance during the first weeks after the operation might receive an explanation A test of this hypothesis was planned as follows since excess of sugar in the blood is more quickly removed after section of the right vagus, it might be expected that the hyperglycæmia, resulting from injection of adrenaline, would in that case also be of shorter duration Two experiments were, therefore, done in which 5 c c adrenaline (P D and Co) diluted 1 in 4 was injected subcutaneously before, and 2 weeks after cutting the right vagus, and the blood-sugar estimated every half-hour for 5 hours It will be seen from Table V that the average hyperglycæmia is essentially the same in the two conditions At first sight this may appear to oppose the theory of tonic inhibitory fibres to the islets, for, after section of the vagus, the readier supply of insulin would be expected to combat more efficiently the hyperglycæmia following adrenaline It is obvious, however, that the secretion of insulin depends on an adequate blood-supply to the islets, and there is no evidence that adrenaline has not the same constrictor action on the vessels of the pancreas that it has elsewhere The greater secretion of insulin, therefore, that is otherwise available after right vagotomy may be held in check by diminution of

TABLE V

Rabbit	Hours after adrenaline	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	$3\frac{1}{2}$	4	$4\frac{1}{2}$	5	Ar increase
12	{ Before vagotomy	100	152	198	237	226	217	209	188	135	114	105	078
	{ After "	114	170	229	234	230	234	230	196	163	122	103	075
13	{ Before "	115	185	239	291	306	300	295	280	236	198	161	134
	{ After "	114	186	258	301	310	325	335	300	233	166	132	140

the blood supply to the islets due to the action of adrenaline on the vessels

Incidentally it may be noted that the results given above do not support the view of Snyder, Wells and Culley(8) that the vagus contains fibres which exert an inhibitory influence on glycogenolysis, for, if their suggestion is correct, an increased hyperglycæmic response to adrenaline would be expected after section of the vagus

The presence in the right vagus of both inhibitory and secretory fibres to the islets of Langerhans would offer an explanation of the failure of earlier workers(2, 3) to produce consistently a hypoglycæmia on stimulating this nerve Britton(4) was able in every case to cause a lowering of blood-sugar by electrical stimulation, but observes that "in a few experiments a brief latent period with slight or no decline in the blood-sugar level following stimulation was sometimes observed" Moreover, in his experiments, the initial sugar level was abnormally high, and it is to be expected, therefore, that any agency tending to reduce this level to normal (i.e. stimulation of secretory fibres) would predominate over one which might raise it still further (i.e. stimulation of inhibitor fibres) That the two sets of fibres are not equally stimulated by drugs is suggested by a comparison of the action of pilocarpine and of guanidine, both of which produce a fall in blood-sugar by vagal stimulation if ergotamine has been previously given to paralyse the sympathetic(1) If degree of salivation be taken as an index of parasympathetic stimulation, it has repeatedly been observed that 1 mg per kilo of pilocarpine will produce profuse salivation but a moderate fall in blood-sugar, while 1 gm per kilo guanidine hydrochloride causes little or moderate salivation but a considerable fall in blood-sugar These facts suggest that pilocarpine may stimulate both sets of fibres It has previously been shown(9) that atropine will paralyse the secretory fibres to the islets, and the results given in Table VI suggest that this drug also paralyses the inhibitory The figures given indicate the response of two animals to the last of four glucose injections given at two-hourly intervals, in one case each injection being preceded by 25 mg atropine sulphate intravenously It will be seen that where atropine has been given the

TABLE VI

Rabbit	Hours after fourth injection	0	$\frac{1}{2}$	1	$1\frac{1}{2}$	2	$2\frac{1}{2}$	3	Fasting B S
14	{ Without atropine	126	227	155	134	127	122	116	{ 105
	{ With "	104	211	147	102	100	100	101	
15	{ Without "	127	242	166	153	145	131	125	{ 103
	{ With "	103	233	149	100	102	100	—	

rabbit shows increased sugar tolerance. The experiments indicate that normally the inhibitor fibres play a more important part than the secretory.

Experiments are being carried out on other lines from which a decision may be obtained.

For the second and later result of section of the vagus when the quickened recovery from hyperglycæmia gives place to one that is unduly delayed, some other factor must be responsible. Observations that point to the influence of the pituitary being here concerned will form the subject of another paper.

SUMMARY

1 After vagotomy the sugar tolerance is for some weeks increased, and not diminished. If the nerve fibres to the islets in the vagus were entirely secretory nerves this would not be expected. The possibility of the vagus containing also fibres that exert a tonic inhibition upon the islets has been considered.

2 This increased tolerance after vagotomy gradually declines and is later followed by diminished tolerance. The explanation for this phenomenon is being investigated.

I wish to express my thanks to Professor Leathes for much helpful criticism in this work.

The expenses of this research have been defrayed by a grant from the Medical Research Council.

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OBSERVATIONS ON THE EFFECT OF ANOXÆMIA UPON HEART AND CIRCULATION

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AND H WASTL (*Research Student of Girton College, Cambridge*)

(*From the Physiological Laboratory, Cambridge*)

STARTING from the observation that heart dilatation sometimes occurs at high altitudes Takeuchi⁽¹⁾ recently correlated the degree of dilatation of the heart with the oxygen content of the arterial blood in cats. He recorded the changes in heart size by using the cinematograph method. Prof Barcroft suggested that we should repeat the experiments cardiometrically.

Methods. In order to vary the oxygen content in the air breathed we devised an arrangement for mixing air with nitrogen.

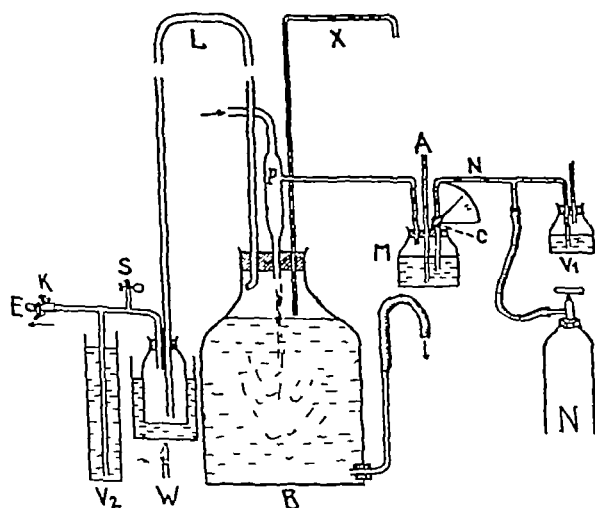


Fig 1

P is a filter pump. *M* is a mixing bottle with about 5 cm. of water at the bottom. *A* is a straight glass tube opening to the air and ending about 4 cm. under the surface of the water. *N* is a similar glass tube reaching about 2 cm. under the water surface, and provided with a cock *C*. The other end of it is connected by means of a T tube with a nitrogen cylinder with a fine adjustment valve and a safety valve *V*₁ which regulates the nitrogen pressure to about 2 cm. of water.

If the filter pump is turned on and cock *C* is fully open pure nitrogen will be sucked through, the pressure of nitrogen being 2 cm of water higher than the pressure of the air in tube *A*. If now cock *C* is gradually closed and the nitrogen supply to *M* diminished, air will bubble from the lower end of *A* into *M*, the proportion of mixture between air and nitrogen being dependent on the position of cock *C*, which can be read on a scale calibrated empirically. The mixture is driven by the filter pump into the large bottle *B*, from which the water escapes from the outlet at the bottom through a rubber tube whose end can be adjusted at a variable height. The gas mixture leaves *B* by tube *L*, a tall band of composition pipe reaching almost to the ceiling, and passes into a small warming bottle *W*. This arrangement is adopted in order to prevent droplets of water from being carried over to the animal. *X* is a small glass tube of about 5 mm bore and 50 cm height, which reaches about 8 cm into the bottle *B*. If more gas mixture is driven into the bottle *B* then leaves it at *L*, the surplus will escape through *X*. *S* is an outlet for taking samples of inspired air and *V*₂ is a safety valve regulating the pressure of inspired air as required. *E* is connected with Takeuchi's (2) revolving cock. The tap water on its way through the pump gives up part of its dissolved gases to the gas mixture, which in this way acquires a constant CO₂ content of 7 p.c., but this in any case cannot be but advantageous to the animal. Some oxygen is added out of the water and this together with the oxygen content of the commercial nitrogen makes it impossible to obtain mixtures with less than 2-3 p.c. of oxygen. From this point up to 21 p.c. of oxygen any intermediate oxygen concentration can be quickly supplied as required in the course of the experiment.

We used cats and rabbits, since we could not employ volatile anaesthetics we gave in most experiments a large dose of urethane (about 1.5 gm per kg), but in some also chloralose or luminal.

We used Rothberger's cardiometer with Starling's (3) rubber cuff, and a piston recorder for registration of the heart volume. Both vagi and phrenic nerves were cut. The blood-pressure was taken from the carotid artery and blood samples from the femoral artery (each sample about 1.5-2 c.c.) with a pipette moistened with a concentrated sodium oxalate solution containing a trace of sodium fluoride. The blood samples were kept under paraffin. The oxygen percentage saturation was determined with Barcroft's differentiator, the volume percentage CO₂ in the blood with Van Slyke's constant volume apparatus using 2 c.c. for each determination. Inspired and expired air was analysed with Haldane's apparatus.

The experiments were conducted in the following way: the required concentration of oxygen was turned on, samples of inspired air, blood and expired air taken as soon as changes in the tracings occurred, or, at the higher values of oxygen content which had no apparent effects, after a corresponding time. With low oxygen concentration, when the heart began to fail, we switched over to ordinary air as quickly as possible in order to enable repeated tests to be made. Therefore our limits are threshold limits in acute anoxæmia.

Respiratory conditions and blood gases The respiratory conditions of

our experiments are illustrated by three examples in Tables I-III (see Appendix) Figs 2, 4 and 5 show the percentage oxygen saturation of the blood corresponding to the oxygen tensions of inspired and expired air. From Fig 2 it will be seen that the curve lies somewhat lower than

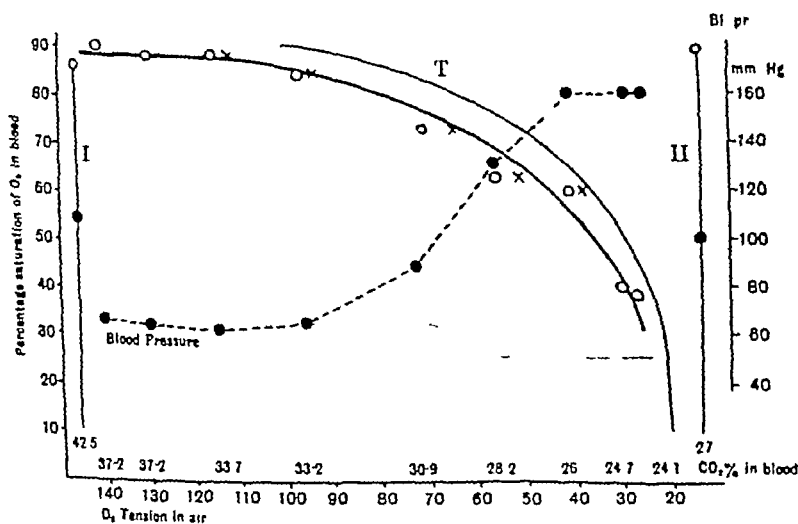


Fig. 2. Rabbit, urethane narcosis chest unopened, vagi cut. Relation between oxygen pressure in inspired air \circ and expired air \times and oxygen percentage saturation in the arterial blood and the blood pressure \bullet , the line signifies the blood pressure in the intervals between the anoxic periods. The volume p.c. CO_2 are noted under the respective points of the curve I and II give the values with natural respiration before and after the period of artificial respiration. T, dissociation curve of rabbit's blood at 36°C and about 40 mm. HgCO_2 pressure (Barcroft and King (4)). It should be observed that the figure does not give any information on the sequence of the different observations but only the values obtained in the different tests in the course of the experiment.

the oxygen dissociation curve of rabbit's blood obtained *in vitro* by Barcroft and King (4) (at a CO_2 pressure 40 mm), but it should be observed that the percentage saturation is not plotted against the alveolar oxygen tensions as it was not possible to determine these. But throughout the experiment the pressure of inspired air and the rate of breathing were constant, and as the vagi were cut, the dead space can be regarded as constant for each experiment. A further circumstance which tends to depress the curve will be mentioned in the remarks at the end of this paper.

The CO_2 content in the expired air is comparatively low as a result of the inevitable over-ventilation with artificial respiration. Corre-

spondingly, the volume p c of CO_2 in the blood drops with the onset of the artificial respiration, the degree of acapnia varying in the different experiments, but the extent of acapnia increases regularly towards the end of the experiment (Decreasing CO_2 production, emptying the CO_2 stores (Haldane⁽⁵⁾), appearance of acid products) This acapnia has to be taken into account in considering the question of the oxygen utilisation by the heart

The hæmoglobin content of the blood, and consequently its total oxygen capacity, decreases as a rule in the course of the experiment These changes can be related to the loss of blood by the taking of the samples and to the general low blood-pressure

Blood-pressure The blood-pressure was generally low in our experiments (70–80 mm Hg) From Fig 2 can be seen that with the onset of the artificial respiration the blood-pressure drops from 110 to 75 mm This is doubtless due to the depressing influence of artificial respiration and acapnia upon the vaso-motor centre (Yandell Henderson⁽⁶⁾, Dale and Evans⁽⁷⁾), for when the artificial respiration was stopped after three quarters of an hour the blood-pressure rose again as high as 100 mm

The response of the vaso-motor centre to anoxæmia in Fig 2 is a considerable rise of blood-pressure beginning at an oxygen percentage saturation between 85 and 73, and becoming maximal at 60 p c saturation

In the above experiment the chest was closed but in experiments with open chest and cardiometer the anoxæmic reaction of the vaso motor centre is somewhat different The rise of pressure is either much smaller or entirely absent or is replaced by a fall In 9 out of 13 experiments the former was the case, in 4 a fall of blood-pressure was observed at such degrees of anoxæmia as produced rise of blood-pressure in the 9 This occurred before the heart began to be impaired (as shown by the cardiometer curves), indicating that the fall of blood pressure was of central origin

The state of the centres is very much impaired by the conditions necessarily involved in the method employed, namely, hæmorrhage, cooling (which cannot be avoided perfectly in cardiometer experiments of long duration), muscle injuries, heavy doses of non-volatile narcotics, and so on, all factors which—as is known—depress the bulbar centres We tried to test the general excitability of the vaso motor centre by stimulating the femoral nerve and the central end of the vagus at the beginning of each experiment and could evoke indeed only a very moderate rise of blood-pressure with very strong stimuli Our exper

ments confirm the view of Mathison(8) that paralysis of the vaso-motor centre occurs very early in asphyxia (Fig 3), and this is quite

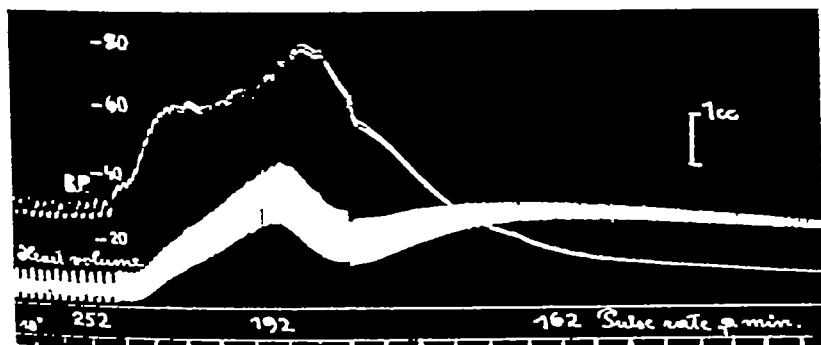


Fig. 3. Shows conclusively that in asphyxia the vaso motor centre becomes paralysed, before the heart fails. On the end of an experiment (cat, under urethane) the artificial respiration was stopped. The heart volume first increases, then decreases, corresponding to the rise and fall of blood pressure. The failure of the heart is signified by a fresh increase of heart volume

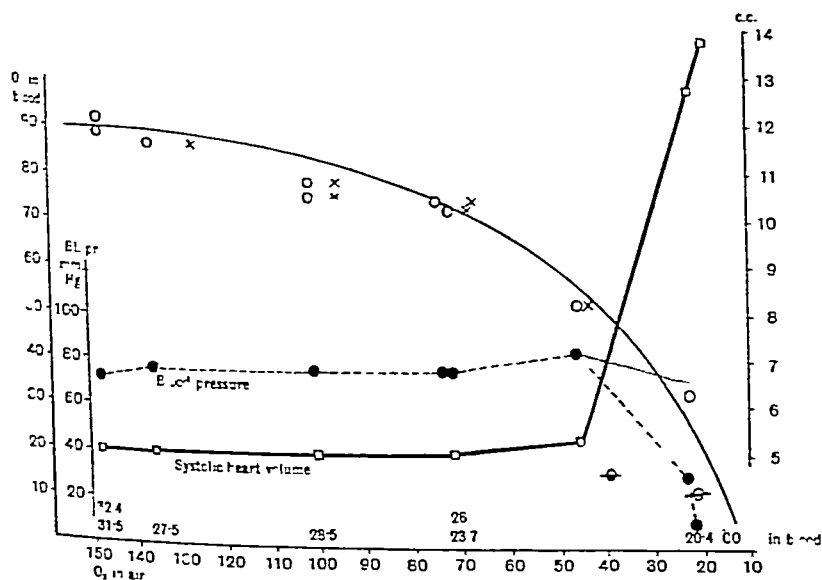


Fig. 4. Relation between oxygen pressure in inspired air \circ and expired air \times and oxygen percentage saturation in the arterial blood and the blood pressure \bullet --- \bullet --- The line signifies the blood pressure in the intervals between the anoxæmic periods \square — \square signifies the systolic heart volume

intelligible as the percentage utilisation of oxygen in the blood is very much lower with nervous tissue than with muscle tissue (L Hill and Nabarro(9)) The tendency for paralysis of the vaso-motor centre was greater in cats than in rabbits

Heart volume Since the heart volume depends upon the blood-pressure we may consider it as a fortunate event for our purpose, that the vaso-motor centre did not play a more prominent part

For simplicity's sake we have given in Fig 4 an experiment where no rise of blood-pressure occurred. The heart volume does not alter before the percentage saturation has fallen below 60 p c. With about 50 p c saturation acute dilatation of the heart sets in, the heart volume rushing up, the blood-pressure dropping rapidly. Quick administration of air restored it to its previous state. With 40 p c saturation the disaster was precipitated. Fig 5 gives an example where a percentage

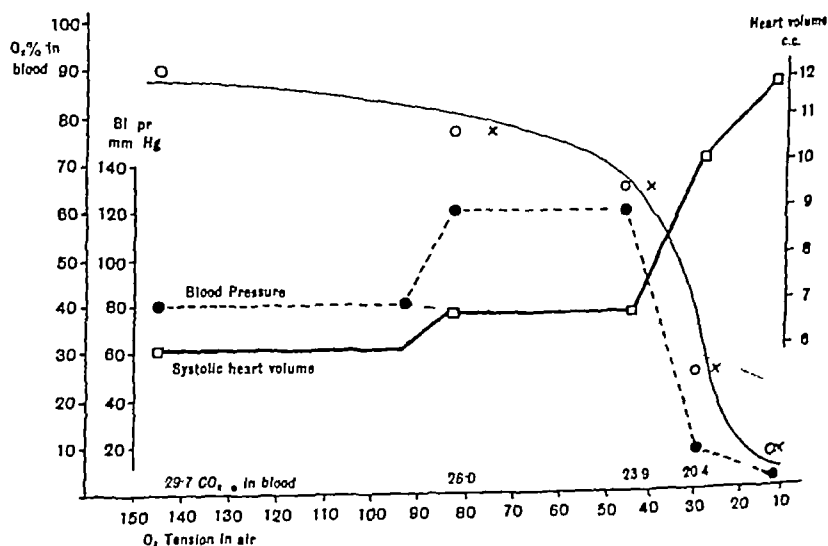


Fig 5 O x ● , □ — □. As Fig 4

saturation of 77 and 65 caused a rise of blood-pressure, with a consequent increase of the heart volume. The disastrous dilatation of the heart only occurred with a further decrease of oxygen supply.

In another experiment in which the blood-pressure was low and responded with further fall to anoxæmia, the result was the same in principle, but dilatation occurred earlier, i.e. when the oxygen content of the blood was not reduced so much. The tracing showed that this

dilatation occurred later than the fall of blood-pressure. It is obvious that any reduction of the coronary circulation due to low blood-pressure—the oxygen percentage being already low—must impair the oxygen supply to the heart muscle and thus produce heart failure.

This leads us to consider the mechanism which produces complete failure of the heart in our experiments, as soon as the heart dilates beyond the mere compensatory dilatation corresponding to the rise of blood-pressure, showing that the heart muscle itself is affected. Obviously we have here a vicious circle establishing itself, namely, that the heart as soon as it becomes weaker impairs its own oxygen supply, multiplying the initial cause of its failure.

Our experiments therefore show that there is a definite critical degree of anoxæmia up to which the heart is not affected and beyond which complete failure of the heart, ending in death, occurs. This critical limit lies at 60 to 50 p c saturation and the available oxygen is still less, due to the Bohr effect as a result of the accompanying more or less pronounced acapnia. This limit appears to be somewhat higher in cats than in rabbits. Green and Gilbert⁽²¹⁾ find as the critical limit of the O_2 content in the inspired air 3.26 p c, they diminished the O_2 contents of the inspired air gradually and observed the blood-pressure and electrocardiogram. These results agree quite well with our observations.

Pulse rate and amplitude. The pulse rate (on the average about 220 per minute, vagi cut) is comparatively little affected by anoxæmia. Degrees of anoxæmia which produce heart failure decrease the pulse rate about 15–20 p c or may leave it unchanged or may even slightly increase it.

Since the pulse rate is scarcely altered the discussion of the amplitude is simplified. We find the amplitude somewhat increased during anoxæmia with rise of blood-pressure. Patterson, Piper and Starling⁽¹⁰⁾ proved this to be the consequence of an increased coronary flow which is added to the ordinary inflow and outflow of the heart when the blood-pressure rises. Besides which there acts in the sense of increasing the coronary flow the tendency of any tissue to open its channels in response to the call for oxygen, in the case of the heart Barcroft and Dixon⁽¹¹⁾ could measure directly the increase of coronary flow in anoxæmia. If the anoxæmia causes vaso-motor paralysis and the circulation as a whole fails, then of course the amplitude of the heart beat diminishes. Table IV gives the corresponding values for every 20th second of an anoxæmic period with 45 mm O_2 tension in the inspired air and 45 p c saturation of the blood.

TABLE IV

Blood pressure (mm. Hg)	Amplitude* c c	Heart volume* increase in c c	Pulse rate per 20 '
92	2.47	0	54
92	2.47	0	50
94	2.47	0.2	55
109	3.07	0.24	62
98	3.07	0.4	66
83	2.93	1.07	56
80	2.53	1.2	51
74	2.05	1.73	52
51	1.87	2.53	53
43	1.84	3.07	48
34	1.73	3.43	44

* Calculated from the calibration curve of the piston recorder

Conclusions It can be deduced from our experiments that the acute dilatation and failure of the heart under want of oxygen already described by Kaya and Starling (12), and Mathison (1c) occurs when the percentage saturation was lowered to about 60 to 50 p c. In agreement with that, heart failure occurred in the experiments of Barcroft and Dixon (11) when they let the animal breathe an oxygen-nitrogen mixture containing about 4 p c of oxygen. The heart seems, therefore, to be very resistant to lack of oxygen in such acute experiments provided that there is adequate elimination of its metabolites. The heart goes on beating even if perfused with oil (Sollmann (13), Guthrie and Pike (21)) or indifferent gases such as nitrogen or hydrogen (Magnus (14)).

Taking into account the conditions of our experiments the actual resistance of the heart muscle ought to be estimated yet higher. (1) The pericardium has been removed, which in itself tends to increase the oxygen need of the heart as proved by Evans and Matsuoka (15), and to decrease its resistance towards factors leading to dilatation (Yas Kuno (16)). (2) The comparatively low blood-pressure due to the impaired action of the vaso-motor centre lessens the efficiency of the important self-regulation protecting the heart, namely the fact that the asphyxic stimulation of the vaso-motor centre raises the blood-pressure by constricting the systemic vessels whilst the coronary vessels dilate, thus increasing the oxygen supply at the most important spot. This is the reason why in the experiments quoted above (Fig. 2) high blood pressure was maintained at percentage saturations which in the cardiometric experiments were far beyond the critical limit.

Now we should like to suggest why our researches differ from the work of Takeuchi (1), who found the chief changes in heart size to occur at the less extreme ranges of anoxæmia. The low pulse rate of his experiments seemed to us to prove that the vagi had not been cut, which is

of importance for the reaction of the heart under anoxæmic conditions, as was shown by Green and Gilbert(20) We therefore made an experiment with vagi intact to test the influence of the vagus reflexes upon the course of the experiment (Artificial respiration, tracheal cannula, anoxæmic stimulation of vagus centre) In this experiment we found, as the only one out of 15, a rise of blood-pressure already with 15.8 p c oxygen in the inspired and 14.15 p c oxygen in the expired air, while the percentage saturation was 72 (this latter value being in agreement with our other experiments as regards the critical limit for the excitation of the vaso-motor centre) Since we had immediately before with 20.9 p c oxygen in the inspired and 18.7 p c oxygen in the expired air, a percentage saturation of 90, and immediately afterwards with 14.8 p c oxygen in the inspired and 12.7 p c in the expired air (without any vaso-motor reaction) 80 p c saturation, the only possible explanation is that it was caused by bronchospasm due in some uncontrollable way to the vagus nerve Further complications arose from irregularities, caused by heart block. If in Takeuchi's film the heart was caught in just such a state, the average value for the heart size will appear to be increased, since by omission of heart beats the size of the heart is increased abnormally during the prolonged diastole

The dilatation of the heart due to anoxæmia is of the type of paralysis such as Socin(17) produced with depressing drugs, and cannot be applied to the question which arose from the observation of increased heart size in man at high altitudes This observation may better be approached from the standpoint of the problem of the tone of the heart A possible analogy to the observations in Peru may be found in the greatly increased hearts of soldiers returning from the mountain front which did not show signs of serious heart muscle weakness, these enlarged hearts decreased in size with short rests only, or quicker after digitalis treatment (Kaufmann and H. H. Meyer(18))

Remarks on the relations between oxygen percentage saturation of the blood and state of general circulation Whilst limiting the critical oxygen percentage saturation for the heart, we tested in one and the same experiment several times the same oxygen concentration in the inspired air We found, without any exception, that the corresponding oxygen percentage saturation of the blood was lower later on in the experiments, the general conditions of the circulation as indicated by the lower average arterial pressure being impaired The correspondence between oxygen percentage saturation and blood-pressure appeared in one experiment in which the blood-pressure was rising spontaneously from 42 to 90 mm.,

and the percentage saturation correspondingly rose from 82 to 90 p c (breathing ordinary air with 7 p c CO_2) Looking for an explanation why the slowing down of the circulation tends to depress the oxygen saturation curve of the blood, one has to remember that the tissues under anoxæmic conditions may pour into the blood acid metabolites which render the blood meionectic (Barcroft and Orbeli(19)), and that the regulating function of the kidney is very much impaired under the same conditions

SUMMARY

1 A gas mixing apparatus is described which delivers mixture of nitrogen and oxygen in any required proportion between 21 and 3 p c of oxygen

2 In cats and rabbits the relation between the oxygen percentage saturation in the arterial blood and the oxygen content in inspired and expired air was determined

3 The vaso-motor centre responds when the percentage saturation is lowered to about 75 p c Usually, rise of blood-pressure then occurs, but sometimes fall of blood-pressure was observed

4 Down to 60 to 50 p c saturation the heart itself is not affected under the conditions of our acute experiments (i.e. with vagi cut) At this critical limit acute dilatation and failure of the heart is imminent

5 Impairment of the circulation decreases the amount of oxygen in the arterial blood

We wish to thank Prof Barcroft for suggesting this work to us and for his kindly interest during its progress To Prof Langley our thanks are due for offering us the hospitality of his laboratory We are indebted to Mr T R Parsons for reading our manuscript

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APPENDIX

Tables I-III are examples to illustrate the respiratory conditions and blood gases. The figures—as far as breathing with 20.9 p c. of oxygen—are such as given by Takeuchi (2) for optimal conditions for artificial respiration with chest open.

TABLE I. Rabbit, urethane narcosis

Inspired air		Expired air		Oxygen percent- age saturation (blood)	Volume CO ₂ p c. (blood)	Oxygen capacity of blood
O ₂ p.c.	CO ₂ p.c.	O ₂ p.c.	CO ₂ p.c.			
20.94*	0.03	—	—	93.0	—	13.7
11.74	0.62	10.54	1.54	75.0	29.74	13.6
6.63	0.70	6.00	1.05	65.0	26.02	13.7
5.48	0.71	3.88	1.14	25.2	23.94	13.4
20.93	0.70	—	—	95.0	20.04	13.2
1.91	0.75	1.70	1.07	7.0	—	13.2

TABLE II. Rabbit, urethane narcosis

Inspired air		Expired air		Oxygen percentage saturation (blood)	Volume CO ₂ p c in arterial blood	Total oxygen capacity (c.c. of 100 c.c. of blood)
O ₂ p.c.	CO ₂ p.c.	O ₂ p.c.	CO ₂ p.c.			
20.95*	0.03	—	—	92.2	32.5	—
20.93	0.70	—	—	89.0	31.5	—
19.20	0.76	17.80	1.95	86.8	27.6	17.5
14.50	0.65	13.50	1.82	79.4	28.5	17.1
14.45	0.70	13.50	1.50	76.0	—	16.6
10.51	0.72	9.60	1.61	77.2	26.0	19.3
10.16	0.78	9.82	1.17	73.1	23.8	19.3
6.75	0.67	6.25	1.02	53.0	—	16.5
2.92	0.63	2.15	1.19	33.3	20.5	15.5
5.50	0.65	—	1.19	15.8	—	15.0
3.12	0.70	—	—	12.5	—	13.5

* Natural respiration, before opening the chest

TABLE III Cat, urethane narcosis

Inspired air		Expired air		Oxygen percent- age saturation (blood)	Volume CO ₂ p c (blood)	Oxygen capacity (total)
O ₂ p c	CO ₂ p c	O ₂ p c	CO ₂ p c			
20.94*	0.03	—	—	80.0	33.85	12.8
20.80	0.70	17.85	2.23	85.0	32.70	12.4
17.15	0.73	15.96	2.25	74.9	30.98	11.1
14.50	0.74	11.50	2.24	71.9	31.85	10.7
10.05	0.72	9.50	1.82	66.0	29.89	10.5
7.56	0.69	6.80	1.40	46.0	25.25	9.8

* Natural respiration, before opening the chest

THE EQUATION EXPRESSING THE EXCRETION OF
A DIURETIC AND ITS RELATION TO DIFFUSION
PROCESSES BY E J CONWAY AND F KANE

Physiology Department, University College, Dublin

I

IN a previous communication(1) it was shown that in the rabbit after the injection of glucose the excretion of this substance by the kidney could be expressed by the formula $\sqrt{V}(C_u - C_B) = K_1$, where V is the volume of urine in unit time and C_u, C_B the concentrations of glucose in the urine and plasma. This formula applies beyond a plasma concentration of 0.43 p.c. (approximately 0.2 p.c. for the whole blood) and beyond a urine volume of about 0.05 c.c. per minute per kidney in a rabbit of 2 kilos. It is applicable at the highest figures obtained for plasma concentration (1.8 p.c.) and for urine volume (3.25 c.c. per minute). The average concentration of urinary glucose at 0.05 c.c. per minute was 10.8 p.c., at 3.25 c.c. it was 2.75 p.c. To produce this very high figure for urine volume a large injection was required and the plasma concentration reached the high percentage value of 1.55.

A similar formula $\sqrt{V}(C_u - 1.25C_B) = K_2$ was found to apply to the excretion of sodium chloride, when the blood concentration was brought well above the threshold value by injections of the order of 50 c.c. of 2 p.c. saline. The minimal rate for this formula is somewhat higher than with glucose, being about 0.09 c.c. per minute. Below this rate the value of K_2 falls rapidly, the urine concentration remaining largely constant. The percentage sodium chloride in the urine is then approximately 1.45 p.c. with an average plasma figure of 0.70.

It was thought desirable to extend the application of the formula to urea when the blood figure is very considerably increased by intravenous injections of urea in saline.

In relation to the temperature effect on urea some experiments had been previously done with urea injections(1), but the urea in the blood was not estimated. In the first experiments now reported the rabbits were anaesthetised with urethane (2 grm. per kilo). This was later changed to veronal (0.6 grm. per kilo) for with urethane the urea concentration of the urine was occasionally well below its average maximum.

value at low urine rates. The intravenous injections used were 5 p.c. urea in 0.6 p.c. saline. The injection was usually about 70 c.c. for a 2-kilo rabbit but the amount was often much varied. The urine was collected from a bladder cannula over short periods of time (1-15 minutes). Before fixing the cannula in position the urethra and adjoining portion of the bladder were ligatured off as well as the blood vessels running along the bladder wall. The cannula was then inserted with its opening close to the entrance of the two ureters and tied in this position, so that very little dead space was present. As a rule two samples of urine, a few cubic centimetres or more in volume, were collected, one a few minutes after the injection, the other towards the end of the diuresis. The fall in the rate of urine as the diuresis passed off was occasionally accelerated by bleeding the animal. During the collection of the urine blood samples were taken from the carotid.

Often at the end of such an experiment an injection of saline was given to determine its effect upon the urea excretion. This effect will be considered later.

The urea in the urine was estimated by Youngburg's modification of the method of Van Slyke and Cullen(2), but smaller volumes were used in the initial removal of the ammonia by permuted lime. The blood urea was determined by a similar method in filtrates prepared by the method of Folin and Wu(3). Controls were carried out with standard urea solutions. The results are shown in Fig. 1 where the values of

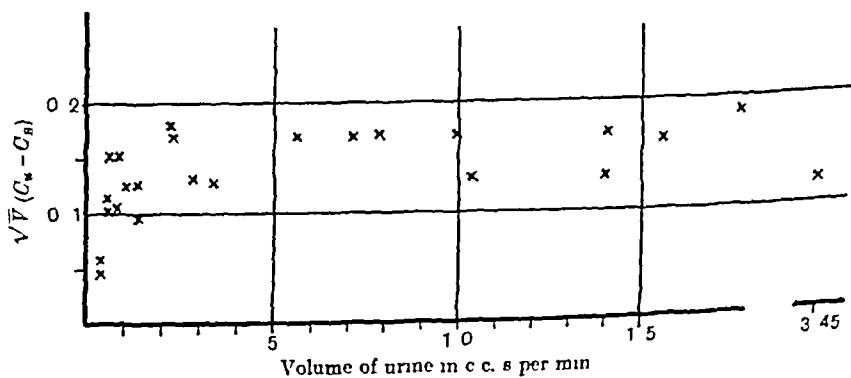


Fig. 1

$\sqrt{V}(C_u - C_B)$ from 14 rabbits are plotted against the urine volume. Beyond a rate of about 0.1 c.c. per minute the points run horizontally and lie around a mean value of 0.15.

The values of $\sqrt{T}(C_u - C_B)$ have been corrected in each case for a standard weight of 2 kilos and a body temperature of 37°C . The urine volume was considered as proportional to the body weight and the value of $\sqrt{T}(C_u - C_B)$ as increasing 10 p.c. for a rise of one degree⁽¹⁾. Many of the urine samples were collected below a rate of 0.1 c.c. per minute. As stated above, it was found subsequently that this figure is the lower limit of application of the formula. Consequently these values are not strictly useable, though as can be seen from Table I the discrepancy

TABLE I.

Exp	Vol. of urine c.c. per min. referred to a body weight of 2 kilos	Body temp	P.c. urea in urine	P.c. urea in blood	$\sqrt{T}(C_u - C_B)$ (corrected for temp)
1	1.56	36.6	1.19	0.45	0.16
	0.33	36.7	1.64	0.32	0.13
	0.08	36.7	3.52	0.36	0.15
2	3.45	37.7	0.78	0.38	0.12
	0.11	36.4	2.74	0.35	0.12
3	1.03	33.8	1.39	0.83	0.13
	0.05	35.2	3.23	0.71	0.11
4	0.78	35.6	1.44	0.42	0.17
	0.07	35.8	3.47	0.33	0.15
5	1.40	35.8	0.89	0.31	0.13
	0.06	37.1	2.82	0.28	0.10

is in many cases not marked, and as will be noticed from the table the range of rate is very wide. The urea concentration can therefore be expressed by a formula similar to that used for glucose and sodium chloride.

The average constant K_3 for urea with 14 rabbits was 0.15, with a corresponding plasma figure 0.07M. For glucose with nine rabbits where corrections for weight and temperature could be applied the average value of K_1 is 0.126, a figure practically the same as the average value 0.130 obtained from experiments on 33 rabbits before the influence of temperature was examined but in which the rabbits were kept warm. The average plasma concentration was 0.037M. For sodium chloride with eight rabbits after injections of the order of 50 c.c. of 2 p.c. NaCl the average value of K_2 was 0.0285. In these C_B had the average value of 0.12M, with no considerable fluctuations from this figure.

As already mentioned, the formula for glucose begins to apply at a plasma concentration of 0.43 p.c. or 0.024M. In the case of sodium chloride it would seem that the formula may apply immediately beyond "the

threshold," but owing perhaps to interferences from other excreta the urinary chloride does not invariably rise to its maximum value at the lower urine volumes unless the blood chloride is considerably increased by the injection of saline. In the urea experiments described above the blood concentration was always above 0.2 p.c. The formula applies beyond this point, but the lower limit was not determined.

II

In another communication(4) the effect of various substances on a glucose diuresis was examined. It was shown that the constant in the formula then used, $\sqrt{V} C_u = K_1$, was considerably decreased following injections of 30 c.c. of phosphate, sulphate, etc. isosmotic with the blood. The expression $\sqrt{V} C_u$ gives approximately the values of $\sqrt{V} (C_u - C_B)$ where the urine volume and plasma concentration are not very high as then the value of C_B is small compared with C_u . The decrease of the excretion constant then noticed was not due to a lowering of the blood glucose, for as many analyses showed this remained well above the concentration beyond which the formula applies. Sodium chloride of 2 p.c. concentration had an effect similar to the other substances. Normal saline (30 c.c.) on the contrary produced comparatively little effect on the urine concentration, i.e. the value of $\sqrt{V} C_u$ tended to rise with the diuresis which, however, was in general slight. The effect of chloride on a glucose diuresis was subjected to a further examination in a series of experiments which we will now consider.

These experiments were varied by using larger injections of normal saline than previously and the urine was analysed for chloride as well as for glucose. Otherwise the procedure was the same, samples of urine and blood being taken during a glucose diuresis before and after the injection of the chloride solution. The results are shown in Table II.

TABLE II

Exp	Saline injections referred to a body weight of 2 kilos	Urine flow after before	P.c. NaCl in urine before saline injection	Increase of p.c. NaCl in urine after saline injection	$\sqrt{V} (C_u - C_B)$ after before
1	25 c.c., 0.9 p.c. NaCl	3.2	0.08	0.03	1.9
2	50 c.c. "	1.2	0.33	0.08	0.9
3	25 c.c. "	2.3	0.02	0.27	0.9
4	48 c.c. "	1.2	0.05	0.35	0.65
Second injections					
1a	50 c.c., 0.9 p.c. NaCl	1.9	0.08	0.18	1.15
3a	42 c.c. ,	2.1	0.02	0.66	0.4
2a	70 c.c. "	2.1	0.33	0.69	0.01

In the table are included the results of three experiments where a second saline injection was given after the fall of the first saline diuresis. In these the increase of urinary chloride, the relative increase of urine volume and value of $\sqrt{T} (C_u - C_B)$ for glucose refer to initial conditions. If there is little increase in urinary chloride concentration the glucose excretion constant tends to rise with the diuresis. This effect will, in general, be produced as was noticed previously by a small injection of normal saline. The resulting diuresis is small and the concentration of glucose in the urine remains practically constant(4).

If the chloride concentration is appreciably increased as by larger injections there is a fall in the excretion constant for glucose and the chloride produces a result similar to that previously shown on injections of sulphate. That this lowering of the constant is not peculiar to glucose as previously surmised(2) is shown by the effect of chloride injections on a urea diuresis. Table III shows the results of experiments similar

TABLE III.

Exp.	Saline injections referred to a body weight of 2 kilos	Urine flow after before	P c NaCl in urine before saline injection	Increase of p c NaCl in urine after saline injection	$\sqrt{T} (C_u - C_B)$ after before
1	70 c.c., 0.6 p c. NaCl	2.1	0.22	0.11	1.33
2	43 c.c., 0.9 p c. NaCl	2.6	0.28	0.14	1.18
3	94 c.c., 0.6 p c. NaCl	4.0	0.32	0.23	0.98
4	49 c.c., 0.9 p c. NaCl	1.0	0.29	0.06	0.92
5	35 c.c.	0.76	0.46	0.31	0.67
6	20 c.c. 2 p c. NaCl	15.0	0.18	0.63	0.22
Second injections					
1a	70 c.c. 0.6 p c. NaCl	12.0	0.22	0.42	0.77
4a	67 c.c., 0.9 p c. NaCl	1.6	0.29	0.52	0.24

to those upon the glucose excretion. It will be seen that where the increase in chloride concentration is very slight the value of $\sqrt{T} (C_u - C_B)$ tends to increase with the diuresis but further increase of chloride causes a corresponding fall in the excretion constant.

Here it may be remarked that diuresis in general increases the chloride concentration in the urine when this has a low initial value(5). This effect, however, is marked only when the urine volume is considerably increased. In these experiments the urine volume with a few exceptions remained comparatively low so that the increased concentration of chloride may be regarded as due to the injected saline and not to an altered rate of urine flow.

The influence of sulphate, phosphate, etc. which had been shown to cause a fall in the glucose constant was not examined but there is no

reason to doubt it would be similar. It would then seem that diuretic substances compete for excretion possibly in relation to their relative concentrations in the tubule cells. This mutual interference of diuretics it may be noted refers only to the excretion constants. The total excretion will largely depend on changes in the urine volume, and this is obviously influenced by the injection of diuretic substances. We do not consider here the factors controlling the urine volume but only the relation between actual rates and concentrations.

III

The formula proposed by Austin, Stillman and Van Slyke⁽⁶⁾ for the excretion of urea in the human subject is

$$K = \frac{D}{B \sqrt{VW}}$$

This formula holds below upper limits of V and B , where

V = Urine volume in unit time,

B = Blood urea,

D = Urea output,

and

W = Body weight,

at uniform body weight this is equivalent to the expression

$$\sqrt{V} C_u = K C_B,$$

where C_u and C_B are the concentrations of urea in urine and blood. It may also be written

$$\sqrt{1/t} C_u = K C_B \quad (1),$$

where t = time of unit volume of urine.

At the upper or, as they term it, "augmentation" limit for the blood (a limit suggested by them from the work of Addis⁽⁷⁾) the formula may be written

$$\sqrt{1/t} C_u = K_a \quad (2)$$

Equation (1) is an empirical relation expressing their own results and the numerous figures of Addis and Watanabe⁽⁸⁾. They find a greater constancy with their equation than that proposed by Ambard⁽⁹⁾. As pointed out in a previous communication equation (1) is related to

$$\sqrt{V} (C_u - C_B) \text{ or } \sqrt{1/t} (C_u - C_B) = K_b \quad (3)$$

considered above. Equation (3) holds much beyond a volume augmentation limit of the order arrived at by them for urea in the human subject.

and applies not only to urea but to glucose, and also to sodium chloride where C_B is altered to $1.25C_B$

It was considered(1) that equation (3) might be related to the work done by the kidney, since the reversible work done in unit time on glucose in a glucose diuresis as calculated from the experimental values did not vary much with the rate. The empirical formula 3, however, approximates much closer to the experimental values than did the minimal work requirement for the glucose excretion. Calculations of the reversible work done on the secretion of urea in the human subject from the figures of Addis and Watanabe(8) give much wider variation than equation (1)

Another and different significance may be attributed to these empirical formulæ. Equations of the same form can be deduced from the diffusion law for simple diffusion. These equations relate the mean concentration C_t in a cylinder with the time t due to diffusion of a solute from a constant concentration C_0 in walls or at one end. Thus equation

$$\sqrt{1/t} C_t = k_1 C_0 \quad (4)$$

represents the case where the concentration in cylinder is zero at zero time

C_0 will have an upper or saturation limit when the equation becomes

$$\sqrt{1/t} C_t = k_2 \quad (5)$$

In the case where C_0 has reached a limit and at zero time there is in the cylinder a uniform concentration C_i small compared with C_0 then

$$\sqrt{1/t} (C_t - C_i) = k_2 \quad (6)$$

It will be seen that equations (4) to (6) have the same form as the empirical equations (1) to (3)

If, however, we consider diffusion to take place into the lumen of the kidney tubule it is a diffusion across an interface from a lower to a higher concentration. We have a parallel for such a process in diffusion of a solute across an interface between two solvents with a high partition coefficient of solubility. We may take as an example the diffusion of iodine from water into chloroform. It is desirable to show that this iodine diffusion is controlled by laws of the same form as those deduced for simple diffusion in a homogeneous fluid

The molecular weight of iodine in water though forming a brown solution is the same as that in chloroform forming a violet solution(10), so that we could expect the partition coefficient at equilibrium to remain constant with changing concentrations of the iodine in water. That the

iodine partition coefficient does remain constant between water and a solvent forming a violet solution is shown by the figures given by Mellor using carbon tetrachloride(11) At a water concentration of 0.1934 the coefficient is 85.51, at 0.0516 it is also 85.51 It is possible within a relatively short period to follow the diffusion of iodine from water into chloroform by the colorimeter up to a point when the total concentration in the chloroform is several times higher than in the water If the observations be started with a solution of iodine in chloroform instead of pure chloroform the diffusion has been followed within a range of total iodine concentration about twenty times that in the water layer In the method used a little chloroform of known depth was contained in one cylinder of the Kober colorimeter The plunger was adjusted to 3 mm from the chloroform surface Down the side of the plunger was attached a capillary tube, bent at the end so that its opening pointed somewhat upwards, being flush with the centre of the lower surface of the plunger Through this capillary tube there passed from a reservoir a solution of iodine in water at a rate of 0.75 c.c. per minute The overflow was drained by capillary siphons The total concentration of iodine in the chloroform layer was estimated at any time t by comparison with standard solutions in the other cylinder The standards were chosen and varied so that similar depths of fluid were compared on each side Table IV gives the figures obtained in a typical experiment

TABLE IV

Depth of chloroform in fixed cylinder = 8 mm Conc. of iodine in water layer = 0.0097 p.c. Temp = 11.5° C

Time in minutes	C_t mean p.c. conc of iodine in chloroform layer	$\sqrt{t} C_t \times 1000$
15	0.0040	1.04
25	0.0052	1.04
32	0.0061	1.08
42	0.0066	1.02
65	0.0073	0.91
77	0.0079	0.90
107	0.0098	0.90
216	0.0147	1.00
273	0.0175	1.06
329	0.0204	1.12
402	0.0219	1.09
426	0.0226	1.09

It will be seen that $\sqrt{t} C_t = K_c$, where C_t is the total iodine concentration in the chloroform after time t

Table V gives the results of varying the iodine water concentration

TABLE V

Depth of chloroform layer = 8 mm

C_w p.c. conc. of iodine in water layer	Temp	$\sqrt{t} C_i / C_w$
0.0024	13.3	0.100
0.0058	12.5	0.095
0.0097	11.5	0.105
0.018	13.0	0.097

The table gives the average values for each experiment. It is clear from the results that $\sqrt{t} C_i = K_d C_w$, where C_w is the iodine water-concentration. This equation has the same form as the theoretical equation (4) for total diffusion and as (1) expressing the urea secretion in the human subject. If the chloroform has initially a uniform iodine concentration C_i , then the following formula experimentally applies

$$\sqrt{t} (C_i - C_i) = K_d C_w,$$

as may be seen from Tables VI and VII

TABLE VI

Depth of chloroform layer = 8 mm. C_i p.c. iodine in chloroform layer at zero time = 0.0125
Temp. = 13.7°C. P.c. conc. of iodine in water layer = 0.0064

Time in minutes	C_i mean p.c. iodine in chloroform layer	$C_i - C_i$	$\sqrt{t} (C_i - C_i)$ (multiplied by 1000)
30	0.0157	0.0032	0.58
55	0.0187	0.0062	0.67
114	0.0194	0.0069	0.65
161	0.0202	0.0077	0.61
212	0.0219	0.0094	0.63
272	0.0228	0.0103	0.63

TABLE VII.

Depth of chloroform layer = 8 mm.

C_0 p.c. iodine in chloroform layer $t=0$	C_w p.c. iodine in water layer	$\frac{\sqrt{t} (C_i - C_i)}{C_w}$
0.0064	0.0058	0.099
0.0125	0.0064	0.099
0.0301	0.0064	0.103

The full expression from theoretical reasons and the analogy of simple diffusion is probably

$$\sqrt{t} (C_i - C_i) = (P C_w - C_i) \times \text{constant},$$

P being the partition coefficient

Here C_t in our experiments is small compared with PC_w and if we considered C_w as invariable or as the saturation concentration of the iodine in water, then

$$\sqrt{l/t} (C_t - C_i) = K,$$

which has the same form as the theoretical equation (6) and as (3) expressing the secretion of glucose, etc

The chloroform layer in the experiments quoted had a constant depth of 8 mm. Varying the depth with each experiment we should expect the relation

$$\sqrt{l/t} C_t l = K, C_w,$$

where l is the depth of the chloroform (This equation is deduced from the diffusion law as applied to simple diffusion) K , was found to be approximately the same at 4, 8 and 16 mm depth. The value of K , at the average temperature allowing for changes in depth up to 16 mm is 0.078. Where there is no renewal of the iodine water the constant falls slowly with the time due no doubt to the depreciation of the iodine water concentration. The initial values approximate to 0.071. If the inflow is considerably increased but maintained steady so that a perceptible motion is given to the water layer the form of the law expressing the iodine diffusion remains unchanged but the value of the constant is increased. It is clear from these observations that the equations expressing total diffusion of a solute across an interface where a high partition coefficient exists between the two solvents have the same form as those expressing similar diffusion in a homogeneous medium.

IV

There is in the facts so far advanced a close parallelism between the excretion of a diuretic and diffusion processes. This is obviously reconcilable with a secretion theory of the kidney but not with a theory which supposes the reabsorption of water. The secretion scheme that first suggests itself is the simple one that all the fluid of the urine comes from the glomerulus and differs little if at all in composition from a blood filtrate and that this fluid is further concentrated by a diffusion secretion process of solutes. If we regard t the time of emergence of unit volume of urine as proportional to the time spent in the tubules then from the iodine diffusion analogy we could expect the two equations

$$\sqrt{l/t} C_u = K C_B$$

and

$$\sqrt{l/t} (C_u - C_B) = K C_B,$$

the first when the blood concentration is low compared with all the urine values obtained, the second when it is appreciably high. Taking urea as an example the two excretion equations are

$$\sqrt{1/t} C_u = K C_B$$

for excretion in the human subject (6) and

$$\sqrt{1/t} (C_u - C_B) = K_3,$$

for the rabbit after injections of urea

It will be noticed that in the last equation which applies when the blood concentration is very high, the right-hand side is a constant and independent of the blood, so that diffusion would in this case appear to take place from a constant concentration in the cells. This assumption, however, does not appear necessary if we consider that within the tubule cells a physico-chemical process is creating the equivalent of a partition coefficient in regard to the diuretic substance between the cells and the urine and that beyond a certain level of intracellular concentration the power of the cell to produce this physico-chemical change has reached its limit. Diffusion might then be expected to take place as if from a constant concentration independent of further increase in the blood. According to this suggestion we could regard the intracellular concentration as varying with the blood and not necessarily higher.

In our treatment of diffusion as it may occur in the kidney an objection can be made to taking the time of unit volume of urine as proportional to the time spent in the tubules as in diuresis the tubules are distended. This distension, however, causes a corresponding diminution in the ratio of surface to volume of the tubule contents. As a result of this an alteration of tubule volume can be shown to be without effect on an application of the diffusion equations.

The excretion of sodium chloride requires special consideration as the value $1.25 C_B$ enters into the formula. According to our scheme this would mean a glomerular fluid more concentrated than the blood. Wearn and Richards' analysis of the glomerular urine in the frog (12) shows approximately the same average relation to the plasma chloride. They consider that the filtration theory as at present defined is not adequate to explain their results, their calculations from the Donnan formula giving insufficient values.

Another point in connection with chloride is that the blood concentration cannot be regarded as small compared with the urine concentration even at very slow rates, and from theoretical considerations

relating to diffusion the formula could be expected to apply better in the form

$$\sqrt{1/t} (C_u - 1.25 C_B) = k_4 (C_{\max} - 1.25 C_B)$$

where C_{\max} is the urine concentration at very slow rates. However, where the value of C_B varies but little in a diuresis or is approximately the same in general as a result of similar injections, this equation becomes that already given.

The simple form of theory used implies that the concentration of any urinary substance cannot fall below its value in the plasma. This of course is not true. The explanation within the scheme advanced might lie either in an absorption of the deficient substance by a reversal of the secretion diffusion process, or in that the glomerulus while in general unable to concentrate is able to retain solutes.

Deviations from the diffusion formulæ as given may be expected under certain conditions which we shall briefly consider. We have shown how the excretion constant of a diuretic (applying at high blood values) is in general decreased by the injection of another diuretic. There seems to be a mutual interference which may be related to the relative concentrations in the tubule cells. This interference will produce a changing value of the secretion constant as determined from the formula and so an apparent deviation from the diffusion processes.

The augmentation limit for the urine volume found by van Slyke, etc. has not been found in our experiments. This may be related to the fact that in our observations the blood concentrations were very high. Also there is the case of substances being formed by the kidney itself in which the blood concentration will be no index of that in the tubule cells. It appears from the work of Behre and Benedict⁽¹³⁾ that we can classify creatinine under this heading.

In conclusion, some speculation may be permitted as to how a partition coefficient can be created in watery media. If we consider that solution in general is related to a combination of solute and solvent and varies with the degree of this combination, then an interference with the hydration of solids in water might produce an alteration in the solution process which would reveal itself as a partition coefficient with water on the other side of a membrane impermeable to the water but permeable to the solutes. One could regard this membrane in the case of the tubule cells as situated only at the lumen side. From such a view the change in concentration of the solute so produced would be largely independent of its chemical constitution. Also, there would be no need to imagine a higher concentration of urea, etc. in the tubule cells than

exists in the blood Since the time of Heidenham such an increased concentration has been held a proof of secretion and conversely its absence a proof of absorption and yet in the case of the liver cells concentrating bile pigments in their secretion there is no evidence of a similar intracellular concentration

SUMMARY

A formula $\sqrt{l/t} (C_u - C_B) = K$ found to apply to glucose and with a modification to sodium chloride applies also to urea when the plasma concentration is much raised by injections In the equation, t is the time of unit volume of urine, C_u and C_B being the concentrations in urine and plasma An equation of the same form applies theoretically to simple diffusion processes and is shown to apply experimentally to the diffusion of iodine from water to a higher concentration in chloroform

A scheme is put forward in which excretion in the kidney may be explained as a similar diffusion process from the walls of the tubules

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THE RELATION BETWEEN THE CORPUS LUTEUM AND THE MAMMARY GLAND

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INTRODUCTION

It is generally accepted that the stimulus for the normal development of the mammary gland is derived from the corpus luteum, the stimulus being hormonal in nature. The question whether the foetus has any influence upon the mammary development during pregnancy, apart from that which it exerts by prolonging the life of the corpus luteum, is, however, still undecided. In view of the intimate connection between this organ and foetal implantation and nutrition, any attempt to solve the problem by removing the corpus luteum, while still retaining the live foetus, seems for the present, out of the question. Loeb and Hesselberg⁽¹⁾ removed the corpora lutea in pregnant guinea-pigs at from 3-6 days after copulation. They state that in some cases an examination at from 14-18½ days after coitus showed that abortion had not taken place, and that a new ovulation, with consequent production of new corpora lutea, had not occurred. It is difficult to reconcile this with their observation that the presence of a corpus luteum is necessary for the production of the decidua, and with Fraenkel's⁽²⁾ statement that, in the rabbit, removal of the corpora lutea in the early stages of pregnancy is always followed by abortion. Loeb and Hesselberg found in the guinea-pig that, whether abortion took place or not, proliferation of the mammary cells, manifested by the presence of mitotic figures, was not taking place at the time of examination. In their control guinea pigs at this stage, of seven cases the mammary cells are described as proliferating in three and as intermediate in four. Proliferation is not then a constant feature of this early stage of pregnancy in this species. Furthermore, associated with the cyclical corpus luteum of oestrus, there is a considerable development of ducts before pregnancy occurs, and the corpus luteum of pregnancy does not appear to stimulate the mammary gland to further growth immediately. The alveolar growth

seems to be started by the action of the corpus luteum of pregnancy—after some retrogression due to the cessation of the stimulus from the previous corpus luteum of œstrus has been made good—at about the time that the cyclical corpus luteum would have degenerated

In the work on the goat described in this paper, no light is thrown on the question as to whether mammary development may occur in the absence of the corpus luteum if the foetus is present, for in all cases except one, abortion followed the removal of the corpora lutea. In the single case which proceeded to term, a small corpus luteum was found near the hilum of the ovary, in which position it had apparently been overlooked at the time of the operation

Woodman and Hammond(3) have shown that in the cow, pregnant for the first time, a cloudy secretion with the consistency of honey is to be obtained from the udder from about the twentieth week of pregnancy onwards (The duration of pregnancy in the cow is about 40 weeks) Asdell(4) confirmed this, and demonstrated the relation of this fluid to colostrum and to milk, colostrum being shown to consist of a mixture of the viscous secretion, often containing up to 38 p.c. of globulin, with milk. The abrupt appearance of this globulin secretion at half-way through pregnancy was shown to occur in the goat also. In this species it appeared at the tenth week of the 21 weeks' pregnancy. This period is known to be that at which the growth of the alveoli, as distinct from that of the ducts, begins. Hammond and Sanders(5) have shown that, at this stage in pregnancy, the yield of the lactating cow shows a distinct fall which is continued until the cow is dry, usually about 8 weeks before parturition. This would appear to be due to the entry of many of the mammary cells upon a growth phase. It is therefore related to the phenomena already described.

In this paper the relation between the corpus luteum, the globulin secretion, and milk secretion is considered. The method adopted has been to remove the corpora lutea or the whole ovary from goats pregnant for the first time. In some cases the operation has been performed about 2 weeks before the globulin secretion was due, in others, 2 weeks after it had appeared. The subsequent mammary reactions have been studied and reported.

Experimental

The modern goat in the hands of the pedigree breeder shows a strong tendency to lactate in the absence of pregnancy (Asdell(4)). The goats in which this phenomenon occurs are nearly always found to be animals with that blend of blood—a blend of English, Nubian and Swiss—which

has produced such extraordinary results so far as the amount of milk yielded in a lactation is concerned. The unimproved native goat does not exhibit this tendency. Accordingly, for the purposes of this work, great care was taken in the choice of animals. Those used were without trace of lopping ears (Nubian), or of tassels and Toggenberg face-markings (Swiss). They were all virgins and were served by the buck under supervision. They were fed in a normal manner.

The corpora lutea were removed from some a fortnight before the thick secretion was due to appear, the latter time being about the tenth week of the 21 weeks' pregnancy. In the others the operation was deferred until the twelfth week, when, except in one case, the globulin secretion had already appeared. The method adopted was to open the abdomen, bring the ovaries to the surface separately, with as little disturbance of the uterus as possible. The latter was not exposed in any of the operations, as it was desired to reduce the risk of operative abortion to a minimum. The corpora lutea were cut into with a scalpel, and shelled

TABLE I *Summary of Operations and their Effects.*

Goat	Effects on foetus	Effects on Mammary Gland	Remarks
<i>Early operations</i>			
1	Abortion, foetuses found	No secretion	—
2	No abortion	2-3 c.c. milk per day, then thick secretion, a few drops each day	Kidded at term, a corpus luteum overlooked
3	Abortion, foetuses found	No secretion before operation, then 1-2 c.c. colostrum each day. The alveoli were developing	New corpus luteum forming
4	Abortion, foetus not found, but blood clot on vulva	No secretion before operation then a little thick secretion. The alveoli were developing	New corpus luteum forming
8	Abortion, foetus not found, but blood clot on vulva	1 drop clear viscous secretion before operation then a few drops of milk, with later a few drops of thick secretion. Globulin demonstrated qualitatively	Whole ovaries removed
<i>Late operations</i>			
5	Abortion, foetuses found	2-3 c.c. thick secretion removed each day before operation then 400 c.c. milk each day. Alveoli well developed	A corpus luteum overlooked. Thick secretion had appeared. Operated at 14 weeks
6	Abortion, foetuses found	Thick secretion at 11 weeks. After operation 300 c.c. colostrum rising to 700 c.c. milk per day. Alveoli well developed	Thick secretion had appeared. Operated at 14 weeks
7	Abortion foetus found	No milk, little development of alveoli	No thick secretion had appeared. Operated at 13 weeks. This appears to be a case of delayed development of the mammary gland

out, the cavity being scraped with a sharp spoon and cauterised. The corpora lutea shelled out readily, leaving a smooth lined cavity. It was found to be difficult to discover all the corpora lutea as they were unpigmented and were sometimes hidden near the hilum of the ovary. If a follicle were pricked during the operation, the cavity was cauterised.

A summary of the operations and their consequences is given in Table I.

Discussion

From these experiments it is evident that the appearance of milk in quantity after the removal of the corpora lutea was always preceded by the appearance of the viscous globulin secretion. The case of goat 7 is of great importance in this respect. It is the only case of a considerable number examined in which the globulin secretion had not appeared by the eleventh week of pregnancy. In this goat, although the corpora lutea were removed at the thirteenth week, no milk was secreted. This exception supports the inference drawn from the other experiments that milk secretion in quantity does not occur unless the mammary gland has passed through a stage of development which is characterised by the production of globulin. It is also shown that the growth of the alveoli is always in progress when the thick secretion has appeared.

In view of the fact that the globulin secretion appeared whether the corpora lutea had been removed or not, in some of the goats operated on early in pregnancy, it was decided to remove the whole ovary in some cases at this period. Unfortunately, circumstances arose which prevented the use of more than two goats for this purpose. Of these, one died after the operation, but the other, goat 8, gave a well-defined positive result. The viscous secretion, demonstrated to be largely globulin, appeared 17 days after the operation, about the normal time for the appearance of the secretion. This suggests that, the stimulus for mammary growth having been given, the gland can continue developing for a time. Either sufficient hormone is stored in the system, or the impetus already imparted to the cells is sufficient for a while. The latter seems the more satisfactory explanation, for it is unlikely that the hormone would remain stored and potent for over a fortnight. There is the possibility that a dead foetus may have been lying in the uterus and have caused this development. This, however, is unlikely, since in goat 3 such a possibility is excluded, while in goat 4 the involution of the uterus was such that abortion of the foetus had apparently taken place a few days after the operation. This development which occurs in the absence of the corpus luteum is not followed by milk secretion.

Further evidence of the importance of the half-way stage in mammary growth is given by Hooper(6), who has considered the effect of abortion at different stages of pregnancy on the milk yield of the lactating cow. In six cases in which abortion occurred in the interval between the 103rd and the 183rd day of pregnancy, *i.e.* up to a month after the globulin secretion would have appeared in a *prima gravida*, the average milk secretion for the month preceding the abortion was 516 lbs, and for the month following 524 lbs. In the second group of six cases abortion occurred from 216 to 254 days after conception. The average duration of pregnancy in the cow is about 285 days. In this group the average yield rose from 411 lbs to 683 lbs. In the earlier set, the rise is of 8 lbs only during the month, while in the later abortions, where mammary growth has set in, the rise is 272 lbs.

Another point which has emerged from this work is that after the removal of the corpora lutea, ovulation is spontaneous, occurring from the sixth day onwards. There are two cases of actual ovulation after operation in these goats, but the signs of oestrus were vague. The spontaneity or otherwise of ovulation in the goat does not seem to have been determined previously, but in view of the cyclical oestrus during the breeding season, it is probable that ovulation always is spontaneous. In order to test this point, a goat was killed 80 hours after oestrus was first observed. Ovulation had occurred, and a corpus luteum was found in each ovary. There was no possibility of coitus having taken place.

The case of delayed appearance of the globulin secretion in goat 7 is of considerable interest for it denotes a delay in the development of the mammary gland. This was confirmed by histological examination. Development was less at 14 weeks than is usual at 10 weeks. Removal of the corpora lutea in this animal did not result in the production of milk as was the rule in goats operated on at this time or a week earlier. This indicates a lack of co-ordination between the corpus luteum and the mammary gland. It is possible that this lack of co-ordination is the result of the action of some unknown inherited tendency. If so, it would furnish an explanation of the action of such genetic factors as are known to affect the milk yield, for the degree of development attained by the gland must determine the maximum yield of milk at the commencement of lactation.

In a paper on the decline of milk yield with the advance of pregnancy, Gaines and Davidson(7) state that this decline is probably due to an inhibiting hormone which is said to circulate in the blood of the pregnant animal, and that this inhibition is removed at parturition. They consider

that the gradual attainment of the maximum yield denotes that this inhibitor is not removed from the blood system immediately after parturition, but persists for a variable time during which the inhibitory effect gradually decreases. The present writers consider that the postulation of an inhibitory hormone is unnecessary. Half-way through pregnancy the corpus luteum secretion stimulates some of the mammary cells to growth. While the cells are growing and dividing, it is unlikely that they can continue to secrete. As pregnancy advances, more and more cells pass into the growth phase, and consequently the milk yield shows a progressive decrease. At parturition or a short while before, the corpus luteum degenerates, and, the growth stimulus being removed, the cells gradually revert to the secretory mode of life.

SUMMARY

Removal of the corpora lutea in the pregnant goat was always followed by the abortion of the foetuses.

If the removal was performed before the globulin stage of mammary secretion was reached, milk was not secreted in bulk. Removal of the corpora lutea after the globulin stage was followed by an immediate copious secretion of milk.

The stimulus for growth having been given to the mammary cells by the corpus luteum, they appear to be able to reach the globulin stage without receiving a continued stimulus. If by excising the corpora lutea, the stimulus is removed, the attainment of this globulin stage is not followed by milk secretion.

Ovulation in the goat is spontaneous.

The influence of the corpus luteum upon the mammary gland is shown to be a variable one, thus providing a possible basis for the action of genetic factors affecting milk yield.

The theory that an inhibiting hormone prevents or causes a decline in milk secretion until parturition occurs appears to be unnecessary.

The writers wish to thank Messrs J. Pike and S. Tedman for their care for the animals under experiment and for their help during the operation.

They are indebted to Dr F. H. A. Marshall for much useful help given during the course of this work.

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THE CORONARY CIRCULATION IN THE ISOLATED HEART. BY M. HAMMOUDA AND R. KINOSITA

(From the Department of Physiology and Biochemistry,
University College London)

THE correct interpretation of experiments on coronary circulation in the whole animal presents so many difficulties that it is necessary to use simplified forms of technique which will permit the experimental conditions to be controlled. Apart from the use of isolated coronary blood vessels the isolated perfused heart and the heart-lung preparation would seem to satisfy the requirements. These two methods have been used by many workers but not always with concordant results. Porter(1) found in the isolated heart that augmentation both in the strength and in the rate of the cardiac contractions increased the coronary flow, Langendorff(2) and Maass(3) that arrest of the heart diminished the flow. Scharrer(4) and especially Wiggers(5) consider that the coronary flow depends intimately upon the massaging influence of the cardiac contraction. On the other hand, Sassa(6) found that acceleration of the heart due to warming the perfusion fluid was accompanied by a considerable diminution in the coronary flow while acceleration produced by means of heating the sino-auricular node caused a slight augmentation of the flow.

Experiments performed on the heart-lung preparation by Markvalder and Starling(7), Nakagawa(8), Hilton and Eichholtz(9) and Anrep and Segall(10) show that neither driving of the heart at different rates, nor strengthening of the cardiac contraction produced by increasing the stroke output have any appreciable effect upon the minute outflow from the coronary sinus. Acceleration of the heart produced by raising the temperature of the blood from 33° to 40° slightly reduces the flow. In the denervated heart-lung preparation, therefore, the arterial blood-pressure is the only mechanical factor which determines the rate of flow through the coronary vessels.

Observations with regard to the action of adrenaline and stimulation of the sympathetic nerve upon the coronary circulation are equally contradictory. Schaffer found no effect. Maass, Sassa, Kravkov(11)

observed a vasodilation Brodie and Cullis(12) observed a vasoconstriction with small doses of adrenaline and a vasodilation with larger doses, whilst Wiggers observed in the resting heart a pure vasoconstriction On the other hand, all workers who used the heart-lung preparation or isolated rings of coronary arteries (Langendorff(13), Pal(14), de Bonis and Sussanna(15), Campbell(16), Barbour(17), Cruickshank and Subba Rau(18)), found adrenaline to cause a pure vasodilation

The experiments on the coronary circulation referred to above have been performed on the hearts of cat, dog, sheep, rabbit and ox It is possible that adrenaline has a different effect in different species of animals, but the contradictory results of the experiments on mammalian hearts cannot be explained in this way, since several workers performed their experiments on the same type of animal

At the suggestion of Dr G V Anrep we have reinvestigated the problem of the coronary circulation in the isolated perfused heart

Method One of the main sources of error in experiments upon the perfused hearts arises, as pointed out by Schafer and by Wiggers, from a part of the perfusion fluid leaking through the aortic valves into the left ventricle, so that all the fluid flowing from the heart does not pass through the coronary vessels Wiggers finds that the magnitude of the leak bears no relation to the coronary flow and that it is necessary to obviate the leak either by inserting the perfusion cannulae directly into the coronary arteries or by draining the left ventricle Wiggers points out another possible source of error, namely, the right ventricle by more vigorous contractions may increase the outflow from the heart as it empties its cavity Ätzler(19) and Sassa, to obviate this error, registered the inflow of the fluid into the coronary blood vessels, while Wiggers and Kravkov worked with arrested hearts The error can also be eliminated by draining the right ventricle either by means of a tube introduced through the auricle or directly through the wall of the ventricle, the pulmonary veins and artery being in both cases ligatured Our experiments (63 in number) were performed on isolated rabbits' hearts, which were perfused through the aorta, or through the coronary arteries In the first case the left ventricle was drained and we found in confirmation of Wiggers that the leak may vary considerably, in our experiments from 8 to 55 p.c. of the total outflow The coronary flow was collected from the right auricle and ventricle, so that both these were empty of fluid Two perfusion fluids were used, (a) oxygenated carbonate-buffered Ringer's fluid, and (b) oxygenated borate buffered

Ringer's fluid (modified Mines solutions⁽²⁰⁾), the second solution being used to maintain the H-ion concentration of the perfusion fluid constant throughout the experiment, and to avoid precipitation of calcium which usually occurs in ordinary Ringer's fluid. Provided the H-ion concentration of the borate-buffered Ringer is maintained within normal limits, it has no detrimental effect upon the isolated heart, no difference in the contractions of the heart, in the coronary outflow or length of survival of the heart being observed.

The composition of the borate-acetate solution used was as follows: boric acid 0.031 p.c., sodium acetate 0.068 p.c., CaCl_2 0.021 p.c., KCl 0.01 p.c., NaCl 0.84 p.c.

The measurement of the coronary flow was made either by collecting the fluid in a cylinder graduated to 0.1 c.c. or by the graphic method employed by Brodie and Cullis. In some experiments this was checked by Ätzler's method of registering the coronary inflow. The perfusion pressure varied between 50 and 110 cm. of water. Records of the heart beat were taken in every experiment. The coronary outflow was studied in hearts beating naturally, in artificially driven hearts, in hearts with ventricular fibrillation, and in arrested hearts; the effect of adrenaline being tested in each case.

(a) *The effect of adrenaline in the naturally beating heart.* Brodie and Cullis found that small doses of adrenaline diminished the outflow, while larger doses caused a diminution which was followed by a prolonged augmentation, the latter phase predominating over the former with an increase in the dose. Two explanations of the temporary diminution in the coronary flow were considered: (1) that it is due to the augmentations of the contraction and to the acceleration of the heart, which produce a compression of the capillaries and thus increase the resistance to the flow until this effect is overcome by the proper dilatatory action of adrenaline, (2) that adrenaline itself in small doses causes constriction, and in larger doses dilation. Brodie and Cullis reject the first explanation on the ground that the diminution of flow begins a few seconds before the augmentation of the cardiac contraction. On the whole our experiments confirm those of Brodie and Cullis, excepting only in respect to the time relations between the onset of diminution in flow and of augmentation of the heart beat. To determine the time relations between the changes in the cardiac contraction and the coronary flow we used two methods, one exactly similar to that of Brodie and Cullis in which the heart was placed horizontally, and another in which the heart was suspended vertically. The results obtained

with these two methods were different. With the horizontally placed heart we obtained exactly the same time relations as Brodie and Cullis, but with the second method we found that the temporary phase of decreased flow either began simultaneously with the augmented contraction or followed it after an interval of a few beats.

Fig. 1 represents an average effect of a medium dose of adrenaline. Very small doses of adrenaline had in the case of the vertically suspended heart, either no effect or a pure diminution in the outflow, in which case an augmentation of the cardiac contraction or an acceleration were also present.

The difference of these results is due to the greater sensitivity of the recording system with the vertically placed heart, when the heart is lying on its side its contractions have to increase considerably before the increase gets registered, since the heart does not lift itself up with each beat and does not exercise a sufficient pull on the lever.

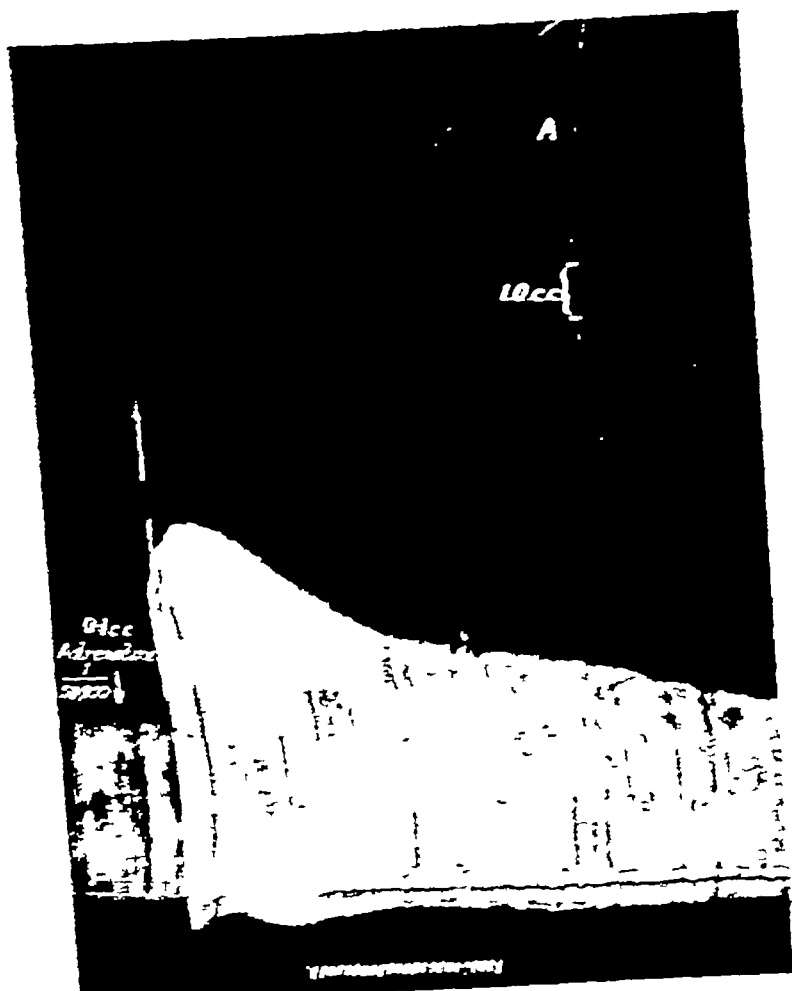
In several experiments the same heart was experimented upon both in the horizontal and vertical positions and we consistently found the results in the first case to be exactly as described by Brodie and Cullis, whilst in the second they were similar to that shown in Fig. 1¹.

The beginning of the augmentation of flow occurs in most cases at the time when the maximum increase in the contractions starts to fall off. The augmentation of the flow lasts for a very considerable length of time, and if all the experiments are taken into consideration it becomes clear that the increased coronary flow bears no relation to the increase in the cardiac contractions.

(b) *Effect of increasing the heart rate and of ventricular fibrillation upon the coronary flow.* The acceleration of the heart beat when produced either by warming the perfusion fluid or by heating the sino auricular node is complicated in the first case by the effect of temperature upon the coronary blood vessels and in the second case by a possible stimulation of the nerves running within the region of the sinus. In our experiments the heart rate was changed by stimulation of the left auricular appendix with rhythmic induction shocks. We found no augmentation of the coronary flow even with considerable acceleration of the heart. In some experiments there was a small and transitory acceleration with the commencement of driving, however, within a few seconds the

¹ In the beginning of their research Brodie and Cullis also used a vertically suspended heart and in these experiments the beginning of the diminution of the flow was definitely later (so far as is possible to judge from their Fig. 1) than the commencement of the increase in the beat.

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after the removal of the current, the coronary flow augments to 56, 70, 86, 88, 84 84 c.c. per minute

The increased coronary flow lasts so long as the fibrillation continues. In cases when the ventricles spontaneously recover from fibrillation the coronary flow diminishes to its previous level.

At the end of an experiment when the ventricles are contracting with diminished strength or stop altogether a short faradic stimulation will also send the heart into fibrillation this will not however, bring about a relaxation of the ventricles which fibrillate in a semi contracted state. In this case fibrillation does not cause any increase in the coronary flow.

The experiments on fibrillation show that the coordinated contractions of the heart do not exercise a greater massaging action upon the coronary blood vessels than does fibrillation.

Injecting of adrenaline into a fibrillating heart causes a definite augmentation of the coronary flow (Exp 5)

Exp 5 Rabbit's heart perfused with carbonate Ringer solution. Perfusion pressure 100 cm. of water, temp. 30°C. Before the injection of adrenaline the coronary outflow was 88-90 c.c. per minute and the heart rate 50 beats per minute.

After injection of 0.2 c.c. of 1:40,000 (readings every 30 seconds)

C flow in c.c. per min.	70	120	134	134	110	126	118	116	100	94	90	88
H rate per min.	80	86	74	74	70	62	62	52	46	46	46	46

The ventricles are now set into fibrillation by a short faradisation, the coronary flow augments from 88 to 102 c.c. per minute at which level it stays for 5 minutes. The same dose of adrenaline is now injected. The ventricles now fibrillate much more vigorously and the coronary flow increases about 20 seconds later.

C flow in c.c. per min.	102	124	144	144	14	132	126	118	110	108	104
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Two outstanding details in the action of adrenaline upon a fibrillating heart should be mentioned, the first is the complete absence of the preliminary period of diminution of the coronary flow which is of nearly constant occurrence in the rhythmically beating ventricle, the second is the almost equal latent period for the dilation to take place in both the rhythmically beating and the fibrillating ventricle. It seems to us that if adrenaline has a true constrictor action which brings about the preliminary diminution in the flow then this constriction should occur in the fibrillating ventricle. The preliminary diminution is more satisfactorily explained by increased resistance to the flow owing to the more vigorous systole compressing the intramuscular coronary blood vessels.

This explanation is strengthened by a few experiments in which the

flow returned to its initial magnitude or sometimes fell even below it (Table I)

Injection of adrenaline into a heart which is driven at a constant rate produces a transitory diminution followed by an augmentation of the coronary flow, the changes are comparable with those observed in hearts beating at their spontaneous rhythm. It thus becomes evident that the augmentation of the coronary outflow cannot be explained by the acceleration of the heart beat, in driven hearts, since adrenaline fails to cause any such acceleration provided of course that the artificial heart rate is above the maximum effect of adrenaline on the heart beating spontaneously.

TABLE I Effect of heart rate upon the coronary outflow per minute

(The heart rate was changed by application of rhythmic induction shocks to the left auricular appendix. The artificial rate was maintained in each case for not less than 5 minutes.)

	Heart rate		Coronary flow c c per min	
	Before	After	Before	After
<i>Exp 1</i>	64	110	6.4	6.4
	66	120	7.0	6.8
	60	180	7.0	6.2
<i>Exp 2</i>	52	80	5.0	5.0
	52	100	5.0	4.8
	50	180	6.0	4.8
<i>Exp 3</i>	40	85	3.2	3.4
	40	100	3.6	3.4
	40	130	3.4	3.0

The experiments given in the above table show that in confirmation of observations on the heart-lung preparation, acceleration of the heart beat within wide limits has no effect upon the minute flow through the coronary blood vessels. If, however, the acceleration is excessive, the coronary flow tends to diminish.

While changes in the heart rate have no effect upon the coronary flow, ventricular fibrillation causes a definite augmentation of the flow. Fibrillation was produced in our experiments by stimulation of the ventricle with a strong faradic current. When ventricular fibrillation is produced early in the experiment the heart fibrillates in a state of nearly complete diastole, the coronary outflow is always in these cases augmented (*Exp 4*). This observation confirms the experiments of Maas and of Hilton and Eichholtz on the dog's heart.

Exp 4 Rabbit's heart perfused with borate Ringer, pH 7.4, temp 38°C, heart rate 50-60 beats per minute. Coronary flow in c c per minute 5.4, 5.4, 5.4. Strong faradic current is applied for 10 seconds to the left ventricle, both ventricles continue to fibrillate.

1 30,000 for 10-15 minutes, (5) perfusion with Ringer containing cyanides

To that we may add a few experiments in which there was observed a spontaneous cessation of contractions. In the case of removal of oxygen or injection of sodium cyanide we observed a quick increase in the coronary flow, thus confirming the observations of Hilton and Eichholtz. With oxygen free solutions a progressive weakening of the contractions was caused.

Administration of Ca-free Ringer, strophanthin and oxygenated saline had no immediate effect upon the coronary flow but after 5-10 minutes when the heart became arrested in diastole the coronary flow was always considerably increased. Fig. 2 shows a case in which the

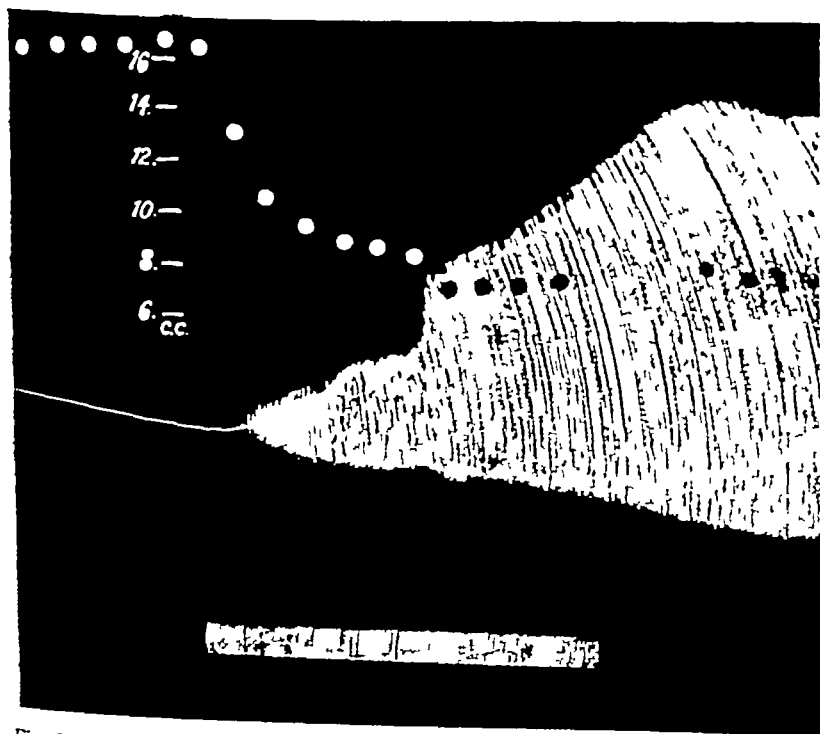


Fig. 2. Effect of diastolic arrest of the heart upon the coronary outflow. The heart is perfused with oxygenated saline of pH 7.4. The coronary outflow is shown by the black and white dots. The tracing should be read from right to left.

heart was arrested in diastole by perfusion with oxygenated saline, in this instance the coronary flow being doubled.

fibrillation of the ventricle stopped spontaneously after adrenaline had been injected (Exps 6 and 7)

Exp 6 Rabbit's heart perfused with borate Ringer's fluid the coronary outflow is registered every 20 seconds, perfusion pressure = 85 cm H_2O , the coronary flow is steady for 5 minutes at 5.4 c.c. per minute. The ventricles are now set into fibrillation by a short faradisation, the coronary flow during fibrillation of the ventricles augments to 6.4 c.c. per minute. Injection of 0.2 c.c. of adrenaline 1:40,000 increases the coronary flow to 6.4, 7.4, 9.8 c.c. per minute. At this stage the auricles begin to beat at 60 and both ventricles beat feebly at 40 beats per minute, the coronary flow falling to 9.6, 9.0, 8.2 c.c. per minute. The ventricles are again caused to fibrillate after the end of the faradisation, the coronary flow is 7.6, 7.4, 7.0 c.c. per minute in consecutive periods of 20 seconds. Two minutes later fibrillation is still present and the coronary flow is 6.2 c.c. per minute.

The effect of adrenaline in this experiment was not changed by the onset of the cardiac contractions. In other experiments in which the contraction started with more vigour the effect was to diminish the coronary outflow (Exp 7).

Exp 7 Rabbit's heart perfused with borate Ringer's fluid perfusion pressure = 60 cm coronary flow = 4.8 c.c. per minute. Ventricular fibrillation is induced by a short faradisation, the coronary flow augments to 5.1, 5.6, 6.4 c.c. per minute. Fibrillation stopped spontaneously and the coronary flow dropped to 4.0, 4.4, 4.5, 4.6 c.c. Ventricular fibrillation is induced for a second time and after the coronary flow reached a steady level of 6.6-7.0 c.c. per minute 0.2 c.c. of 1:40,000 adrenaline is injected.

Coronary flow in c.c. per min. 7.0, 8.6, 9.4, 8.8, 8.4, 8.4, 7.6. The ventricles continue to fibrillate the whole time. After an interval of 10 minutes another 0.2 c.c. of adrenaline is injected. Coronary flow in c.c. per min. 6.6, 8.4, 9.2, the ventricles suddenly recover from fibrillation and beat forcibly at the rate of 75 per minute, the coronary flow abruptly diminishes to 6.0, 5.6, 4.8 c.c. per minute and reaches after an interval of 5 minutes 3.6-4 c.c. a minute.

Injection of adrenaline in the later stages of the experiment, whether into the fibrillating or the beating heart, never produces the same degree of augmentation in the flow as while the heart is fresh.

The doses of adrenaline necessary to produce acceleration and especially augmentation of the contractions are much smaller than those which cause a dilation of the coronary blood vessels. In experiment with very small doses it can be clearly seen that the heart does respond with vigorous contractions and beats faster, but only a pure diminution of the flow can be observed which is not followed by an increase and which coincides with the period of strong cardiac contractions.

(c) *Effect of arresting of the heart in diastole and in systole* The heart was arrested by the following methods (1) removal of oxygen, (2) perfusion with oxygenated or unoxygenated 0.9 p.c. NaCl of the same H-ion concentration (Wigger's method), (3) perfusion with Ca-free Ringer, (4) perfusion with Ringer solution containing strophanthin

that adrenaline caused vasodilation most of those who worked on the arrested heart found a diminution in the coronary flow which was explained by vasoconstriction. However Elliott (20), perfusing quiescent pieces of the cat's heart found that adrenaline caused a vasodilation. Kravkov found also a vasodilation in the rabbit's heart which was arrested by strophanthin no precautions were taken, however to obviate the leak through the aortic valves. Wiggers and Campbell observed a diminution of the outflow which they ascribed to vasoconstriction.

As shown in Fig. 2 a diastolic arrest of the heart is accompanied by a considerable increase in the coronary flow. In this experiment the arrest of the heart was produced by substituting the Ringer fluid by saline both oxygenated and both of pH 7.4. In the cases when the diastolic arrest comes on spontaneously without any modification of the perfusion fluid the sequence of events is exactly the same. However, in none of the cases the coronary flow remains at its new high value

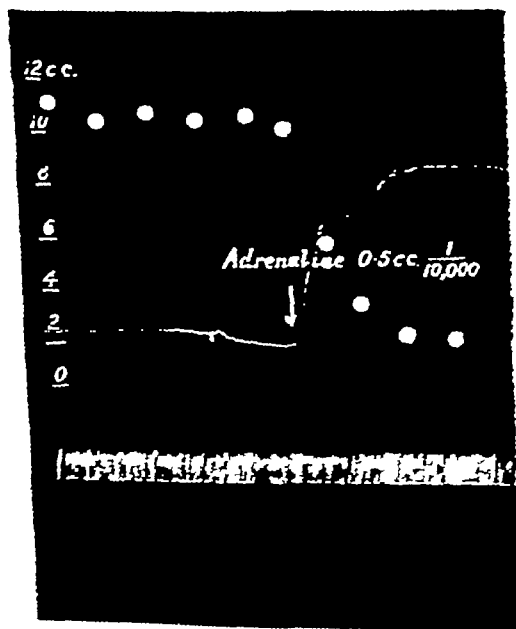


Fig. 4. Effect of adrenaline upon the coronary flow on the arrested heart (white dots). The curve shows the contracture of the heart muscle. The tracing should be read from left to right.

When the heart is arrested in systole whether this is preceded by ventricular fibrillation or not, the coronary flow is greatly diminished. This is illustrated in Fig 3. The heart was being perfused with non-oxygenated Ringer solution and consequently the coronary flow was

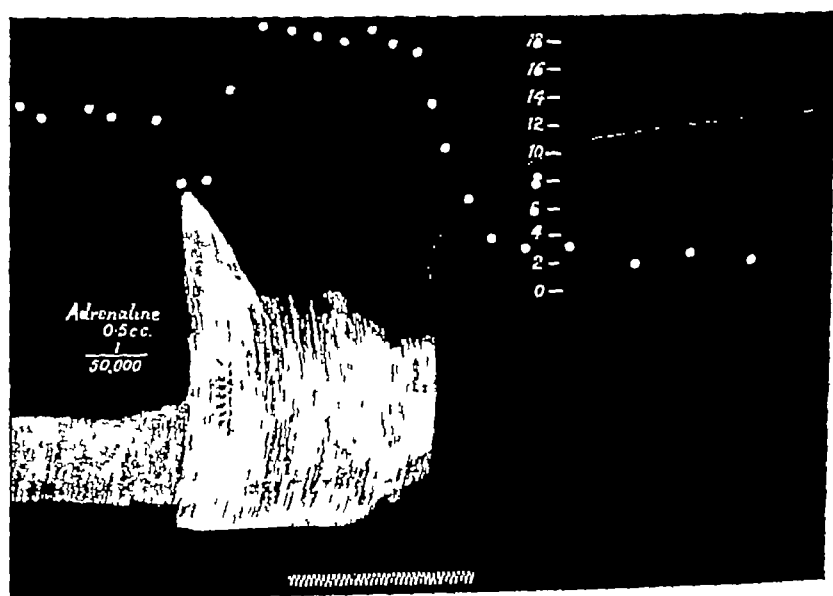


Fig 3 Effect of adrenaline and of systolic arrest of the heart upon the coronary outflow (white dots). The tracing should be read from left to right.

large. A large dose of adrenaline was then injected, which as usual at first decreased and then increased considerably the coronary flow. Suddenly the heart went into a systolic contraction which was preceded by violent ventricular fibrillation, it contracted definitely beyond the maximum height of contraction in the first phase of the action of adrenaline. Simultaneously with this arrest in systole the coronary flow diminished from over 18 cc to about 3 cc per minute. We had several more cases in which the heart assumed such a prolonged systolic contraction, either for no obvious reason or after a large dose of adrenaline, in every case the coronary flow greatly diminished. On the other hand, if the heart was arrested in diastole the coronary flow increased.

We have now to deal with the contradictory observations concerning the action of adrenaline on the beating heart and on the arrested heart. Those authors who worked on the beating rabbit's heart found

that adrenaline caused vasodilation, most of those who worked on the arrested heart found a diminution in the coronary flow which was explained by vasoconstriction. However, Elliott (20), perfusing quiescent pieces of the cat's heart, found that adrenaline caused a vasodilation. Kravkov found also a vasodilation in the rabbit's heart which was arrested by strophanthin, no precautions were taken, however, to obviate the leak through the aortic valves. Wiggers and Campbell observed a diminution of the outflow which they ascribed to vasoconstriction.

As shown in Fig. 2 a diastolic arrest of the heart is accompanied by a considerable increase in the coronary flow. In this experiment the arrest of the heart was produced by substituting the Ringer fluid by saline both oxygenated and both of pH 7.4. In the cases when the diastolic arrest comes on spontaneously without any modification of the perfusion fluid the sequence of events is exactly the same. However, in none of the cases the coronary flow remains at its new high value

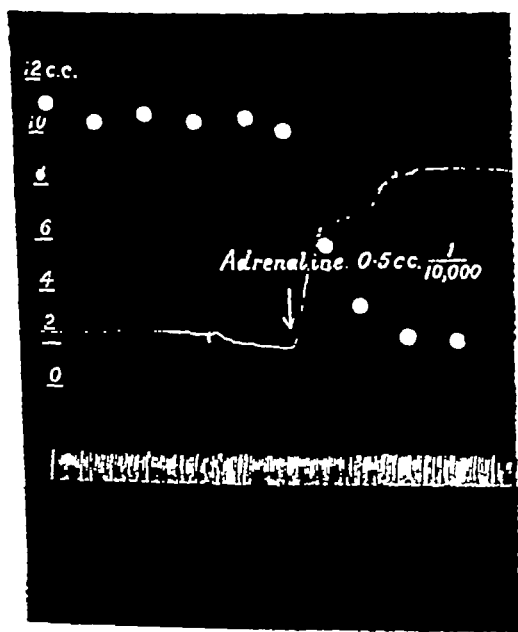


Fig. 4. Effect of adrenaline upon the coronary flow on the arrested heart (white dots). The curve shows the contracture of the heart muscle. The tracing should be read from left to right.

for more than 2-5 minutes, the flow soon begins very gradually to diminish, this period of diminution continues in different hearts for about 15-30 minutes when the flow reaches a minimum. In every case it can be noticed that simultaneously with the diminution of the flow the state of the heart muscle changes also. The heart gradually and spontaneously enters into a state of contraction which finally brings about a greater shortening of the muscle fibres than that observed in the strongest contractions of the beating heart. We are not in a position to say whether this contracture is due to a quickly developing rigor or a real tonic contraction. Adrenaline accelerates the development of this contracture, a case which is illustrated by Fig 4. In this experiment the heart arrested spontaneously, before the arrest the coronary flow varied between 5 and 6 c c per minute after the arrest it augmented to 11 c c, an injection of adrenaline was followed by a contracture of the muscle and a simultaneous diminution in the outflow to about 2 c c per minute. If adrenaline is injected soon after the arrest of the heart and in not large doses, an acceleration of the flow can be sometimes observed, in most cases, however, it is diminished. Fig 5 shows the effect of six successive injections, each followed by a diminution of the outflow. From the second injection onwards the muscle relaxes after the effect of adrenaline has worn off, the coronary flow correspondingly increases.

While the whole heart when arrested reacts to adrenaline by a diminution in the outflow it is easy to show that this diminution is not due to an active compression of the blood vessels, but rather to an increased compression of the capillaries. If a few incisions are made in the muscle so as not to cut any larger branches of the coronaries and the perfusion carried through the arterial side, adrenaline shows a definite dilator effect which is not counterbalanced by the contraction of the surrounding muscles. The blood vessels react in this case in the same manner as isolated rings.

These observations confirm the experiment by Elliott, who worked on strips of cat's heart.

CONCLUSIONS

- 1 Changes in heart rate have no effect upon the magnitude of the coronary flow in the isolated heart.

- 2 Adrenaline has a dilator effect upon the coronary blood vessels of the rabbit.

- 3 The preliminary diminution of the coronary flow is due to mechanical factors, it coincides with the period of maximal contractions of the heart.

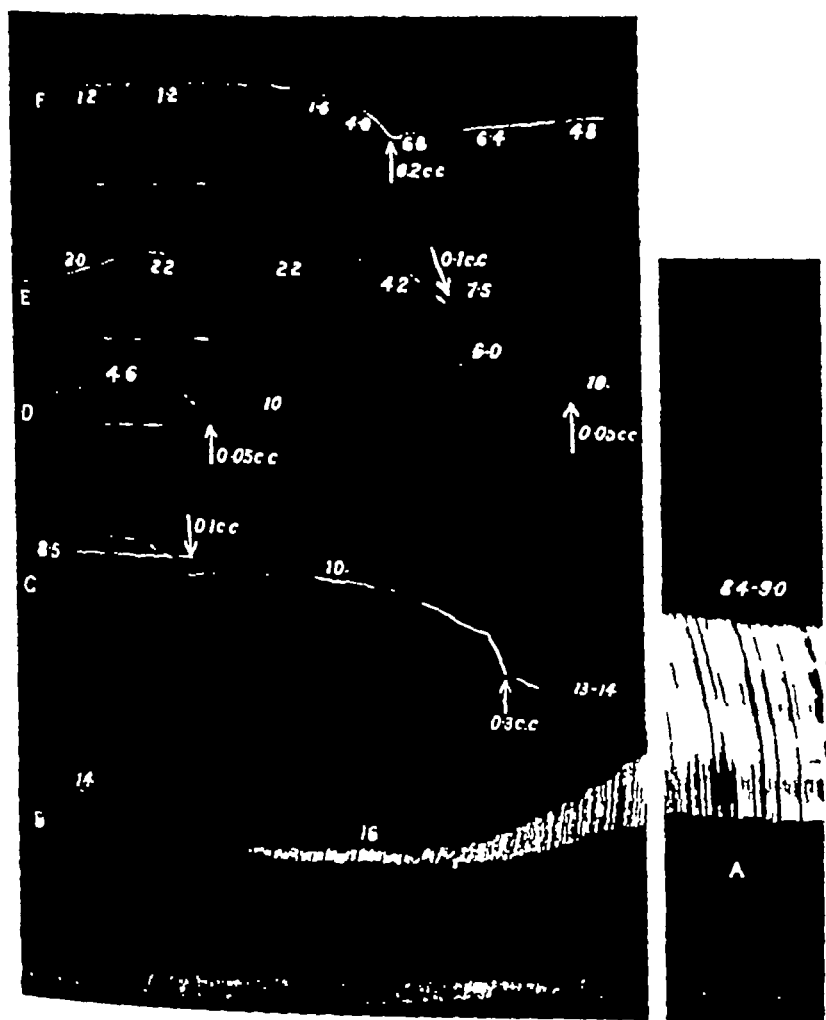


Fig 5 The tracing should be read from right to left A, normal heart beat and coronary flow, B-F, effect of six injections of adrenaline upon the arrested heart. Adrenaline (1:50,000) is injected at the arrows. The magnitude of the coronary flow is shown by the figures on the curve. Tracings B-F are continuations of one another. The four short horizontal lines indicate the height of the normal systolic contractions. It can be seen that the heart enters into a state of contracture which finally becomes more vigorous than the normal systole. The coronary flow progressively diminishes with increase of contracture. Each injection of adrenaline accentuates the contracture and the diminution of the coronary flow.

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PROCEEDINGS

OF THE

PHYSIOLOGICAL SOCIETY,

October 17. 1925

The excretion of phosphate during water diuresis

By R. E. HAVARD and G. A. REAY

Large diureses (500 to 1500 c c per hour) were produced in the normal human subject by drinking water. Samples of urine and blood were obtained at intervals of about 30 minutes. As the diuresis sets in the kidney turns from concentrating to diluting the plasma phosphate which remains constant during the whole experiment. Sulphates and urea, however continue to be concentrated in heavy diuresis. The urinary phosphate may represent as much as a 50 p c dilution of the plasma phosphate. This behaviour excludes phosphate from the category of "no-threshold" bodies, as defined in terms of Cushing's theory of kidney excretion. Mayrs admits the possibility of a very slight reabsorption of no-threshold bodies by the tubules but regards this as mere "renal inefficiency". This can hardly be urged in the case of a 50 p c reabsorption.

If phosphate is a "threshold" body, the actual mechanism which allows the kidney to change over from concentrating to diluting while the plasma phosphate remains steady, is difficult to picture.

On the other hand, we find that during diuresis the diluted phosphate maintains a constant rate of output and thus lends some support to the view of Eicholtz and Starling, that all phosphate is secreted by the tubules. A constant rate of secretion may thus be maintained, which is independent of the amount of filtrate passing the glomerulus.

Capillary permeability By HOWARD FLOREY

The following experiments were performed with the object of ascertaining the precise path traversed by crystalloids and colloids in their passage from the interior of the blood capillaries.

A solution of 0.5 p c pot ferrocyanide and 0.5 p c iron ammonium

4 Fibrillation of the relaxed ventricle increases the coronary flow, the dilator effect of adrenaline can be also shown on the fibrillating heart

5 The diminution in the coronary flow caused by adrenaline in the arrested rabbit's heart is due to a compression of the blood vessels by the muscle and not to vasoconstriction¹

6 The arrest of the heart in diastole is accompanied by an increase and the arrest in systole by a diminution of the coronary flow

We are grateful to Dr G V Anrep for directing this research and for helping us during the experiments

The experiments on the plethysmographic registration of the coronary flow were performed by R K All the rest of the experiments by M H

Part of the expenses of this research was defrayed by a grant from the Ministry of Education, Egypt

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¹ Since these experiments were completed one of us found that adrenaline considerably augments the coronary flow in rabbits hearts which are arrested by arecoline in this case however adrenaline fails to cause any contracture of the cardiac muscle

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citrate was perfused through a loop of intestine (dog), the experiment being arranged so that the perfusion was begun as soon as the blood flow was arrested. Perfusion lasted 4 minutes. 10 p c formalin acidified with 0.5 p c HCl was then perfused. This rapidly fixed the capillary wall and precipitated prussian blue wherever the perfusion fluid had penetrated.

Sections demonstrated that the prussian blue was precipitated within the cytoplasm of the endothelial cell. The nuclei of these cells remained free from deposit. This was taken to indicate that the perfusate passed directly through the cell protoplasm in its exit from the capillary.

Similar perfusions were performed on the rabbit, using 8 p c soluble starch solution. To allow of the escape of the starch two procedures were adopted. (a) After washing out the blood from the selected portion of gut (1-2 min perfusion) the portal vein was tied and the pressure within the vessels thus raised to the perfusion pressure (75 mm Hg). The vessels were fixed in formalin-iodine, the starch being precipitated as the insoluble blue compound. Sections were cut by embedding in gelatine (at 37° C so as to prevent dissociation of starch-iodine compound) and with a freezing microtome. (b) The gut was inflamed by a preliminary injection of abrin (in dog). The capillaries were thus made more permeable than in their normal condition. Starch solution was perfused and sections obtained in the way just described.

The sections prepared in this way showed a precipitate of starch in the cytoplasm of the endothelial cells—this being evenly spread throughout the cell longitudinally but being more copious towards the inner surface of the cell (i.e. the one towards the blood stream).

It would seem that colloids such as starch also find exit directly through the endothelial protoplasm.

Under conditions producing deformation, structures such as gelatine and soap gels become doubly refractive. The effect of stretching gelatine on the rate of diffusion of Hb solution into it was tried. 2-5 p c gelatine was layered 4-5 mm thick over a rubber membrane capable of being stretched. The membrane was tied over the mouth of a large thistle funnel and stretching was accomplished by inflation of the thistle funnel. Each set of experiments was carried out under identical conditions. It was found that diffusion occurred up to 27 p c more rapidly into the stretched than the unstretched gelatine. The increase of surface suffered by the gelatine was about 11 p c, so that there was a real difference in the diffusion rate. It is suggested that the increase of permeability on dilatation of a blood capillary may be partly explained thus. At least

the surface of an endothelial cell may be considered as presenting a gel structure composed of molecules which have one axis longer than the other. On dilatation this structure is stretched—its molecules become orientated and present larger paths for the passage of molecules from the blood stream than exist in the unorientated condition of the molecules. The experiments with the diffusion of Hb into gelatine suggest that the stretch opens up more paths for diffusion.

Further observations on the changes in the adrenal bodies under varying conditions By SWALE VINCENT¹ (Middlesex Hospital Medical School)

I have recently² recorded that if white rats be exercised at laboratory (winter) temperature their body temperature will fall and the chromaphil reaction of the adrenal medulla will be very considerably reduced. If they are fatigued at higher temperatures their body temperature will rise and the chromaphil reaction will not be reduced. At about 18–20° C the temperature of the animal will neither rise nor fall under the influence of fatigue and the chromaphil tissue is unaffected. Reduction of the temperature of the surrounding medium will, independently of fatigue, cause a reduction in the chromaphil reaction if the animal's temperature becomes lowered, which does not always occur. It seems then, that the effect of exercise to the point of fatigue on the chromaphil reaction is not direct, but secondary to the lowering of the animal's temperature.

Further experiments show that if adrenin be injected subcutaneously into the rat half-an-hour before exercise begins, fatigue carried to the point of collapse will not then reduce the intensity of the chromaphil reaction in the adrenal medulla. The body temperature, however, in most cases, falls as usual.

There appears to be a definite correlation between the intensity of the chromaphil reaction in the adrenal medulla and the oestrous cycle. Recent investigations by Stockard and Papanicolaou³ have made it possible, by the study of vaginal smears, to determine precisely what stage in the oestrous cycle the animal has reached. Using this method it is not difficult to satisfy oneself that in the late oestrous and early post-oestrous stages there is a very marked reduction, amounting some-

¹ Towards the expenses of this investigation a grant has been made by the Government Grant Committee of the Royal Society. Much valuable assistance has been rendered by Mr R. H. Pittman.

² *Quart. Journ. Exp. Physiol.* 15, 1925. (In the Press.)

³ *Amer. Journ. Anat.* 22, p. 225, 1917.

times to practical disappearance, of the chromaphil reaction of the adrenal medulla. The stage referred to is that in which large numbers of "cornified" epithelial cells can be recognised in the preparation

Lactic acid and rigor mortis in fish muscle

By A. D. RITCHIE

The lactic acid content of the muscle of three species of *Gadidae* before, during, and after rigor mortis are given below. The fish were caught in the St Croix Estuary, New Brunswick, or in that neighbourhood during June to September 1925, except for two of the Cod caught off Halifax, N.S., in May 1925.

Fish	Lactic acid p.c.					
	Before rigor		During rigor		After rigor	
	Mean	Extremes	Mean	Extremes	Mean	Extremes
Haddock	0.15	0.11-0.17	0.26	0.22-0.35	0.29	0.24-0.41
<i>Melanogrammus aeglefinus</i>	(11)		(10)		(6)	
Cod	0.08	0.06-0.09	0.12	0.10-0.15	0.16	0.11-0.21
<i>Gadus callarias</i>	(2)		(5)		(5)	
Hake	0.05	0.04-0.07	—	—	0.06	0.03-0.09
<i>Urophycis chuss</i> or <i>tenuis</i>	(2)				(7)	

The figures in brackets are the number of experiments performed. The estimations were made by weighing the lactic acid as zinc lactate. The variations within each species are probably not due to age or size differences, as the haddock are at least as variable as the others but appeared to be all of one school spawned in 1920 and were fairly uniform in size. The figures for lactic acid before rigor do not represent the resting minimum, which is about 0.08 p.c. or less for haddock.

The differences between the three species correspond to the usual notion of their muscular activity. The haddock is most active, the hake least.

In muscles which had passed out of rigor, very little more lactic acid could be obtained by chopping with chloroform or keeping for some hours at about 30° C. or even by keeping the chopped muscle in alkaline phosphate solution. This suggests that the lactic acid maximum in these fish, unlike the frog, is due to approximate exhaustion of the glycogen. A rough estimation of glycogen in a haddock, confirmed the suggestion. Muscle containing 0.17 p.c. lactic acid contained also 0.16 p.c. of glycogen, $(C_6H_{12}O_6)_n$, allowing 0.33 p.c. for the lactic acid maximum.

In all these fishes rigor passes off within 12 hours after death at

room temperature in the neighbourhood of 20°C . Confirming the suggestion made many years ago by Halliburton, the passing off of rigor was found to be accompanied by hydrolysis of the muscle proteins. The free amino groups in the whole muscle substance were estimated by Sørensen's Formol Titration method. The method is very rough but the changes found were well outside the experimental error. In four different haddock the mean value for amino nitrogen was equivalent to 7.9 c.c. of normal alkali per 100 gms. at the beginning of rigor. The value increased gradually from the time when rigor was maximal or a little earlier and reached 10.2 c.c. by the time it had passed off. That this effect is due to protein amino groups and not to ammonia or any other soluble base was shown by titrating alcohol extracts (80 p.c. alcohol) in a similar way. With haddock muscle the change is not more than 0.1 c.c. per 100 gms., the total titre of the alcohol extract being about 0.2 c.c. This result may be contrasted with the figures for alcoholic extracts of skate muscle, in which ammonia is produced post mortem. In a skate (*Raja Radiata*) the titre of the 80 p.c. alcohol extract rose from 3.3 c.c. to 5.3 c.c. in 21 hours.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
November 14, 1925

On the survival of striped mammalian muscles By A. HEMINGWAY
and R J S McDOWALL, King's College, London (*Preliminary
communication*)

In 1913, one of us (R J S McD) described certain striped muscles of the hedgehog which were capable of survival for long periods and which contracted even at 0°C . It has now been found that under certain circumstances the ordinary skeletal muscle of mammals can be made to survive for long periods at ordinary temperature. Ordinarily such muscle, even if kept at body temperature and well supplied with oxygen, dies within an hour or so.

The method of preparation consists of anaesthetising the animal, *eg* with chloralose, and injecting intravenously saturated sodium bicarbonate solution. In the case of an average cat, 20–30 c.c. may be injected 2–4 c.c. at a time at intervals of 10 minutes. The animal is subjected to temporary asphyxia from time to time by closing the trachea and after 2–3 hours is bled to death.

Preparations of striped muscle taken from an animal so treated will survive if kept stretched in a moist chamber for 24 hours at ordinary room temperature and for at least 48 hours if kept in ice. The preparations may be used instead of frog's muscle for ordinary class work and are extremely sensitive to changes of temperature.

It has also been found that even if alkali be not injected the tissues of an animal so prepared survive for many hours.

PROCEEDINGS

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PHYSIOLOGICAL SOCIETY,

December 12, 1925

The spleen and the resistance of red cells By D ORAHOVATS
(Preliminary note)

1 The red cells contained in the spleen pulp are less resistant to hypotonic salt solutions than the red cells from the general circulation.

The following data are the average of experiments

Strength of salt solution	68	64	60	56	52	48	44	40	36
Percentage of corpuscles { General }	0	0	5	12.5	40	80.5	90	94.5	98
hemolyzed { Spleen pulp }	0	15.5	30.0	57.5	79	92	96	100	100

On the other hand the red cells of the spleen pulp are more resistant to saponin than those of the general circulation

Strength of saponin (parts in 10,000)	01	1	3	5	7	9	11	13
Percentage of General circulation	3	10	49.0	82	96	99	100	100
corpuscles hemolyzed Spleen pulp	0	0	16	70	90	96	98	100

2 The probable interpretation of the above phenomena in the light of the work of Rywosch(1) is that the erythrocytes of the spleen would yield, on ashing, a less amount of phosphate than those of the general circulation

3 The disparity in resistance between the cells of the spleen and those of the general circulation does not appear to be connected with the length of time during which the cells have been in the spleen pulp, it is as great for cells taken from the pulp immediately after the spleen has contracted and refilled as it is if the spleen has not emptied itself for a long time

4 The statement of Pearce, Krumbhaar and Frazier(2), that the resistance of erythrocytes taken from the general circulation of animals recently splenectomised, is abnormally great has been confirmed

(1) Pwrosch Pfügers Archiv 116 229 1907

(2) P M Pearce, Krumbhaar and Frazier The spleen and anaemia.

Impulses from a single sensory end-organ By E D ADRIAN and
Y ZOTTERMAN

A paper by one of us in the current number of this *Journal* gives records of the action currents set up in sensory nerve fibres when the end-organs are stimulated, but the interpretation of these records is difficult because the number of fibres in action is unknown. We have now succeeded in recording the afferent impulses from preparations containing only a limited number of sensory fibres and in many of these it is possible to detect the impulses set up in a single fibre. The preparation used was the sterno-cutaneous muscle of the frog. The nerve to this muscle contains about 15-20 nerve fibres and there is at least one muscle spindle. When the muscle is stretched, the nerve shows a series of electric responses occurring rather irregularly at a frequency of 100 a second or more. Successive strips of the muscle are now cut away in order to reduce the number of end-organs in action, and with each section the frequency of the responses becomes smaller and signs of definite rhythms appear. Finally the records show one regular series of responses, or two or three regular series of slightly different period. We take each of these regular series to represent the impulses set up by a single end-organ. The frequency of the series ranges from about 10 to 50 per sec, increasing as the stimulus is increased. The magnitude of the responses remains the same whatever the stimulus. By recording the frequencies with stimuli of different strength it is possible to construct a 'recovery curve' for the end-organ. It appears to have the same form as the recovery curve of a nerve fibre, but the rate of recovery is about 5 to 10 times as long. The regular discharge of impulses with a constant stimulus we ascribe to the relatively slow rate of adaptation of the end-organ compared with the rapid rate of adaptation in a nerve fibre which prevents a constant stimulus from setting up more than one impulse. The adaptation of the end-organ has been measured by various methods. A full report will be published shortly.

The sensitising action of alkalis By A HEMINGWAY,
King's College, London

Experiments have been performed in which the effect of rapid transient changes of hydrogen ion concentration have been made in the fluid perfused through the hind limbs of cats. The perfusing fluid

was Ringer's solution, but without NaHCO_3 , and adjusted to a P_H of 7.3-7.5

A rapid change of P_H to the alkaline side by the addition of $\frac{n}{100}$ NaOH or saturated NaHCO_3 , gives rise to momentary vaso-constriction, and the reaction becomes increased with each succeeding change of alkalinity

Simultaneously with this there is an increasing reaction to drugs

The effect is apparently due to the OH' ion, since it may be brought about by KOH with the same facility as with NaOH

Oxygenation of the solution and the duration of the experiment do not influence the onset of the reaction

Similar results have been obtained on perfusing the pulmonary vessels and more recently on the isolated uterus

The spleen as a reservoir for blood and hæmoglobin

By E. W. H. CRUICKSHANK

Barcroft and others(1) have shown by models from X-ray photographs that the spleen contracts when the animal is exercised, asphyxiated or bled, and in virtue of this contraction supplies a definite amount of blood to the general circulation. I have corroborated this by measuring accurately the amount of blood expelled upon contraction of the spleen, and have further determined to what extent the spleen tends to concentrate the blood which it holds. In carrying out these experiments it was essential that the spleen should be retained unexposed in its normal environment. This was done by an operative procedure which exposed only the coeliac axis and the junction of the splenic, superior mesenteric and portal veins. The blood supply of the organ was temporarily suspended, the splanchnic fibres accompanying the splenic artery stimulated with a weak faradic current, and the blood issuing from the vein was collected in a 10 c.c. microburette. By means of a Jaquet clock the rate of flow, and thus the rate of contraction, of the spleen was determined. The hæmoglobin content of the blood per c.c. was determined calorimetrically and at the end of the experiment the weight of the contracted spleen was taken.

The conclusions arrived at are as follows

The spleen is a reservoir for blood since it can in contraction expel up to twice its P.M. weight of blood the amount of blood added to the

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The spleen is a reservoir for blood since it can in contraction expel up to twice its P.M. weight of blood the amount of blood added to the

general circulation varies from 2 to 6 p c of the total blood volume of the animal

In acting as a reservoir for blood, the spleen incidently is a reservoir for hæmoglobin, but strictly its action in this respect is limited. The degree to which it acts as a storehouse for hæmoglobin is measured by the extent to which it can concentrate the large amounts of blood which it is capable of holding. This concentration is maximal about the third c c expelled and here only does it amount to a 40 p c increase. For the whole amount of blood expelled upon contraction, the increase in hæmoglobin over and above the normal content per c c of blood amounts to not more than that amount of hæmoglobin which is contained in 1 or at most 2 c.c. of the blood of the animal under observation.

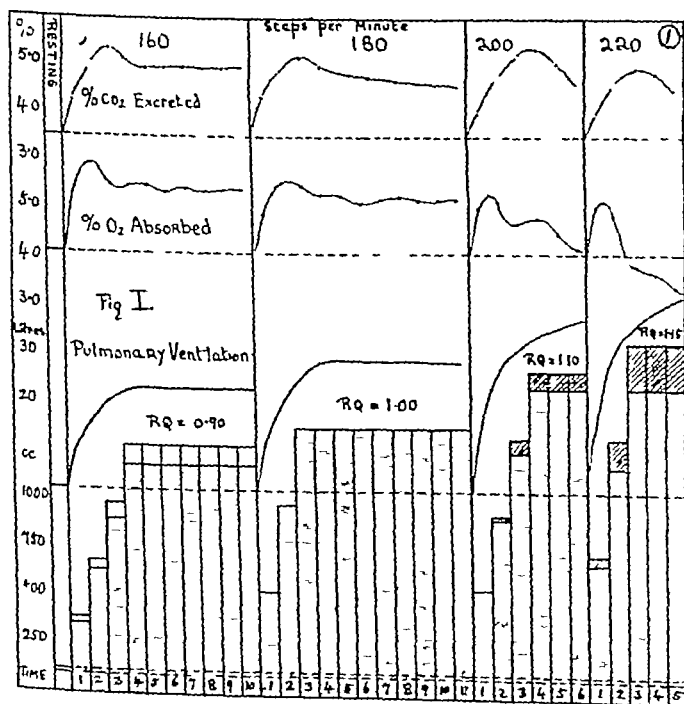
By means of the method employed, a curve of the contraction of the spleen can be obtained. The velocity of contraction of the organ is maximal during the expulsion of the first 5 c c of blood, after which it steadily decreases. The period for the spleen to attain maximal contraction may be of the order of 5 to 10 minutes.

(1) Barcroft, Harris, Orahovats and Weiss. *Journ Physiol* 60 443 1925

The effect of breathing carbon dioxide on muscular exercise in man By A E CLARK-KENNEDY, H N BRADBROOKE and T OWEN

The circulation is usually looked upon as the important factor in the limitation of muscular effort. Thus A V Hill⁽¹⁾ has attributed this to accumulation of lactate when the maximum circulatory rate fails to meet the demand for O₂ supply, CO₂ output was not considered. But severe exercise does not lead to the venous congestion that characterises heart failure, suggesting that the normal heart as opposed to the diseased can always cope with the venous return. Also, vital capacity is reduced in compensated heart disease, and Peabody⁽²⁾ has here attributed the limitation of effort to diminished capacity to breathe. Clinically we have come to regard the respiratory mechanism rather than the circulation, and CO₂ elimination rather than O₂ intake, to be the important limiting factors in muscular effort. Reduction in vital capacity would then limit the work done, and so keep the venous return within the reduced capacity of cardiac output, congestive failure only supervening when the limits of this protective reaction were reached. Thus could be explained the difference between physiological exhaustion and heart failure, and that between compensated and decompensated heart disease.

Fig 1 shows semi-diagrammatically the respiratory exchange during standing running at four different rates. At the two highest, the steady state had been passed, and the O_2 intake was maximal but the CO_2 output and pulmonary ventilation continued to rise. The RQ suggests rise of c_H due to accumulation of lactate. The rise in the percentage CO_2 curves suggests increased H_2CO_3 and $BHCO_3$ content of the blood at the beginning of exercise, and their subsequent fall decreased H_2CO_3 and $BHCO_3$ content due to accumulation of lactate. The pulmonary ventilation has then to rise to keep the output of CO_2 constant. Consequently



less O_2 is taken out of unit volume of inspired air and the alveolar O_2 tension rises to reach its maximum at complete fatigue. Therefore, during hard work the elimination of CO_2 becomes progressively more difficult because the accumulation of lactate, (a) by raising c_H demands a greater CO_2 output, (b) decreases the carrying of the blood for CO_2 , and (c) lowers the tension and percentage of CO_2 in the alveolar air, necessitating more pulmonary ventilation than before to eliminate unit quantity. On the other hand, the rising alveolar O_2 tension must favour oxidation of hæmoglobin, and rising c_H its reduction in the tissues. We,

therefore, studied the effect of breathing 5 p c CO_2 and found that it causes retention of CO_2 during work, but reduces the maximum O_2 intake only 10 p c or so, and probably limits the maximum O_2 debt that can be incurred

Rise of c_{H} is probably important in causing the subjective distress which limits voluntary effort. We suggest that the limits of the steady state, and the size of the maximum O_2 debt, are determined by an equilibrium between O_2 intake and CO_2 output, dependent upon the partition of available base between the two acids, lactic and carbonic. High O_2 tensions, by slightly increasing, and low by decreasing the saturation of the blood, shift the equilibrium by respectively delaying and accelerating the accumulation of lactate. The former by favouring CO_2 elimination lowers c_{H} and this allows a greater O_2 intake per minute and for the same limiting rise of c_{H} a larger debt. The latter by hindering CO_2 elimination raises c_{H} and thus only permits a smaller O_2 intake per minute, and for the same limiting rise of c_{H} a smaller debt. Such a theory makes the lungs an important factor in the limitation of effort, but demands that the rate of removal of lactic acid at constant O_2 pressure falls off with rise of c_{H} .

- (1) Hill, Long and Lupton *Proc Roy Soc. B* 97 p 155 1924.
- (2) Peabody and Wentworth *Arch. Int. Med.* 20 p 443 1917

PROCEEDINGS

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January 23, 1926

The influence of the vagus on sugar tolerance

By G A CLARK

The right vagus has been shown to contain secretory fibres to the islets of Langerhans(1,2,3,4) After section of the right vagus in the neck, no alteration in glucose tolerance was apparent when 1 gm. of glucose per kilo in 10 pc solution was injected intravenously in the rabbit as described in an earlier paper(3) In further experiments, however, a definite increase in glucose tolerance has been found which lasts for 2-3 months after vagotomy, in these experiments four intravenous injections of 1 gm of glucose per kilo were given at two-hourly intervals and the blood-sugar estimated every half hour after the last injection The blood-sugar curve of the normal animal shows a definite lag under

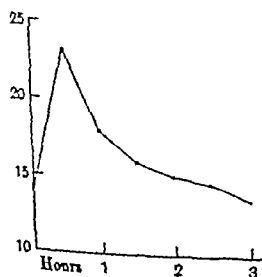


Fig 1

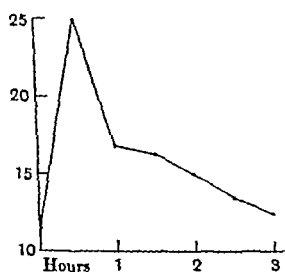


Fig 2

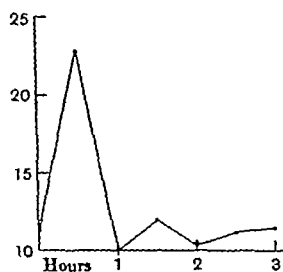


Fig 3

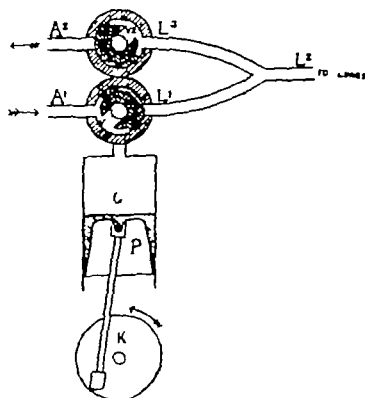
these conditions (Fig 1), while that of the animal after vagotomy returns abruptly to the original level (Fig 3) The initial sugar values appear high because the hyperglycemia due to the third injection has not subsided at the time of determination In all cases the vagus was cut below the origin of the cardiac branches Section of the recurrent laryngeal branch alone in two experiments did not cause increased tolerance (Fig 2), this appeared on subsequent section of the vagus

trunk About three months after vagotomy a second phase appears in which the blood-sugar curve following a single injection of 1 gm of glucose per kilo shows a definite lag, which is still apparent in varying degree 10 months after the operation, indicating a sugar tolerance less than normal

- (1) de Corral. *Zeitschr f. Biol.* 68 395 1918
- (2) McCormick, Macleod and O'Brien. *Trans Roy Soc Can.* 17 57 1923
- (3) Clark. *This Journ.* 59 466 1925
- (4) Britton. *Amer Journ Physiol* 74. 291 1925

An improved method of artificial respiration By ERNEST H STARLING, University College, London

In the ordinary method of artificial respiration there is a side opening to the tracheal tube, through which part of the air escapes during inflation, and the air from the lungs is allowed to escape between each stroke of the pump This method is wasteful of power and of any



volatile anæsthetic employed, and rapidly exhausts any reservoir of a gaseous mixture which it is desired to administer To remedy these drawbacks double acting pumps with two cylinders and valves have been devised by Meyer and by Schuster These, even when accurately made, fail to secure equality of input and output as soon as any differences arise in the resistances to the thrust and exhaust of the pump, so that the lungs tend to become either over-distended or over-deflated and suffer in consequence I have found that the best method is to use a single cylinder pump with solid piston and to introduce valves in the course of the inlet and outlet tubes, so that the lungs receive a

constant adjustable volume of air at each thrust of the pump and are then allowed to deflate by their own elasticity. The arrangement of the pump and valves is shown in the accompanying diagram. The cycle of operation is as follows. On the downward stroke of the piston, P , the cylinder, C , is opened to the air via V^1 and A^1 . When the piston, P , reaches the bottom of its stroke, the valve, V^1 , has turned sufficiently to cut off C from A^1 and immediately connect it to the tube, L^1 . Upon the upward stroke of the piston air is forced from C back through V^1 to the lungs by the tube L^1 , L^2 , thus inflating the lungs. During the downward stroke of the piston, V^2 opens and the lungs become deflated by their own elasticity via L^2 , L^3 , V^2 and A^2 . The valves are actually cut in the axial shaft of the driving cone, K .

With this arrangement the lungs remain in perfect condition throughout experiments lasting four or five hours. The pump serves for the economical administration of gaseous mixtures from a bag or reservoir. On the other hand, a small Krogh spirometer can be placed in the course of the expiratory tube and connected also with the inlet of the pump. By this means working on a closed circuit we may record graphically the actual consumption of oxygen by the animal under artificial respiration, or by a heart-lung preparation.

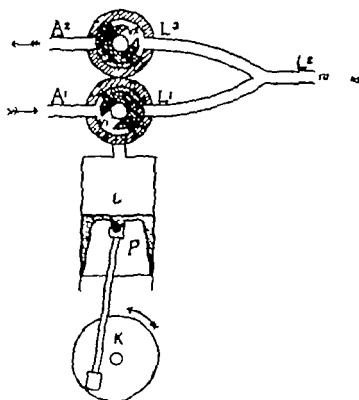
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PROCEEDINGS

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February 20, 1926

The gases of urine and bile By G A. BUCKMASTER
and H R B HICKMAN

Comparatively little information exists concerning the gas-content of the liquids of the body other than blood

The only values known to us are those given by Zuntz (1882), A. Loewy (1911) and Neuberg (1911)

Analyses have been made of the gas-content of absolutely fresh urine and warm bile

Without coming in contact with the air, the liquid was introduced into a tapless blood pump(1), which was of high efficiency, the last traces of air being removed with cocoa-nut charcoal kept at a low temperature

Owing to slight differences in procedure, about 40 experiments have been arranged in series, from which the following short table which only gives data as to the gas-content has been abstracted

	c c.	Total gas evolved in c c	c.c of gas in 100 c.c. N T P		
			CO ₂	O ₂	N ₂
Bile	85 00	12 85	12 67	0 22	1 00
	78 50	22 73	24 9'	0 89	1 95
	174 08	20 43	9 72	0 59	0 64
	174 08	22 93	10 94	0 09	1 10
	174 08	24 08	11 21	0 10	1 03
Urine	174 08	13 93	6 44	0 24	0 90
	174 08	14 35	6 08	0 29	0 84
	174 08	17 25	7 63	0 28	1 42
	174 08	29 40	13 96	0 25	1 53
	103 15	5 70	3	0 63	0 84
	174 08	12 95	5 12	0 33	1 52
	174 08	10 95	4 28	0 46	1 13
	174 08	12 70	5 39	0 23	1 03
	109 00	6 45	4 17	0 39	1 04
	93 00	5 55	4 26	0 28	1 26

The object of the experiments was to ascertain the oxygen content of urine and bile, for if these liquids, as is apparently the case, hold this gas in simple physical solution, then the tension of oxygen may exceed that

of arterial blood, e.g. Pflüger's value of 0.66 p.c. of oxygen in saliva gives a tension of 209 mm. of Hg

The nitrogen values are what might have been expected and show that no air gained access to the pump

(1) Buckmaster and Gardner Journ. Physiol. 43 401 1912

Observations on the effects of temperature and of drugs on the coronary and systemic arteries By E. W. H. CRUIKSHANK and A. SUBBA RAU (*Preliminary communication*)

Comparison of the effect of temperature on the coronary and systemic arteries of the ox The coronary artery is relaxed when removed from the body and responds to temperature by a steady contraction which begins at about 18° C. and reaches a maximum at 37.5° C. With the systemic artery on the other hand the response is one of contraction beginning at about 18° C., but stopping at 27° C., afterwards the artery relaxes steadily while the temperature is raised to 37.5° C., and ultimately reaches a point beneath the original base line

The effect of adrenaline Adrenaline causes a definite relaxation of the coronary artery, thus indicating that the inhibitory mechanism in the coronary is controlled by the sympathetic system. The effect of adrenaline on ergotoxinised systemic arteries depends on the dose of adrenaline, if small, a reversal will be obtained, if large, ergotoxin will be antagonised and a contraction will result. There seems to be in the coronary arteries a definite antagonism between the action of heat on the motor nerve endings, and that of adrenaline on the inhibitory nerve endings

The effect of ergotoxin Ergotoxin stimulates and then paralyses the motor mechanism of the systemic artery. On the coronary it acts in a similar manner, first causing a small contraction, followed by a marked relaxation. The subsequent addition of adrenaline will now cause the artery to relax further. Ergotoxin evidently acts upon that mechanism which is stimulated by heat, i.e. the motor or parasympathetic system

These experiments would indicate that the coronary arteries are supplied with both sympathetic and parasympathetic fibres, the former controlling the dilator or inhibitory mechanism, the latter the constrictor or motor mechanism

The human coronary artery Rings taken from the human coronary artery react to temperature and adrenaline in a manner similar to that described for the coronaries of the ox and dog

A class experiment to demonstrate the effect of load on the frog heart By L S HOBBS, R I HYDER and R J S McDOWALL (King's College, London)

The effect of load on the frog heart may very simply be shown by attaching to an ordinary frog heart lever a paper cone made watertight by dipping in molten paraffin, and by the addition and removal of water with a pipette various loads may be applied without any disturbance to the heart beat. It may be shown that within limits the heart responds to an increased load by an increased response according to the law of the heart. It may also be shown that if the heart be slowed by vagal stimulation a vagus escape may be brought about by an increased external load, an analogous condition to that found in mammals where the increased tension in the right auricle brings about vagus escape.

The relation of the vagus to respiration
By R J S McDOWALL (King's College, London)

It is well known that the classical slowing and deepening of respiration when the vagi are cut are frequently not obtained. This has been shown to be due to the state of the respiratory centre obtaining at the time of the section. Carbon dioxide, lactic acid, small doses of adrenaline ($\frac{1}{10}$ mg repeated) and sensory stimulation affect the respiratory centre to cause a reduction or disappearance of the effect of the vagus. Rest alkalies and ergotoxine, on the other hand, increase the effect of the vagus section, indeed the section may bring about complete cessation of respiration. These effects may be seen in a chloralosed animal and compared by blocking the vagi with a constant current if care is taken to avoid too severe asphyxia, too large doses of adrenaline and the immediate stimulating action of sodium bicarbonate. The results suggest very strongly when taken together with the effect of those procedures in the pupil and heart rate, the control of respiration through the sympathetic-parasympathetic balance.

A convenient method of decerebration
By R J S McDOWALL (King's College, London)

The method consists in sawing through the skull from the angle of the jaw and at a right angle to the lower ramus with an ordinary hack-saw. The line of the brain section is behind the optic thalamus and the tips of the occipital lobes are severed from the cerebral hemispheres. The usual

measures described by Sherrington to prevent hæmorrhage are taken. The advantages of the method are that the operation may be carried out with the animal on the ordinary table, there is little or no shock, and interference with breathing is exceptional, while compared with the trephining operation there is no doubt as to the complete ablation of the cerebrum as the actual point of section is seen.

The formation and permanence of colourless crystals of hæmoglobin of guinea-pig and rat By D FRASER HARRIS

About 25 years ago I prepared some crystals of hæmoglobin from guinea-pig's blood by the method of Stein which consists merely in stirring some blood into some Canada balsam. The blood is not defibrinated or laked. The mixture is "messy", but usually within a few minutes well-formed crystals of hæmoglobin appear and are remarkably permanent.

It was easy to obtain beautiful tetrahedra from the guinea-pig, and hexagons as well as acicular crystals from the rat.

In the preparation demonstrated I noticed that within a week or two, some of the crystals while retaining their sharp outline had become perfectly white, in which state they have ever since remained.

In the case of the rat I have seen colourless crystals form *de novo* from the defibrinated, laked blood.

What exactly is this white substance? It is not "reduced" hæmoglobin, for that is still coloured.

"Bleached hæmoglobin" is, as far as I know, not any substance recognised by biochemists. Again, why should only some of the crystals have become white?

The bleaching—whatever that is—is not a matter of ageing for most of the tetrahedra of the guinea-pig's blood and all the hexagons of the rat's blood, prepared 25 years ago, are still of their original bright red colour.

Some years ago I wrote to *Nature* asking for suggestions regarding the nature of the white crystals, I received only one answer which was to the effect that the crystals were bilirubin. But bilirubin is a pigment

PROCEEDINGS

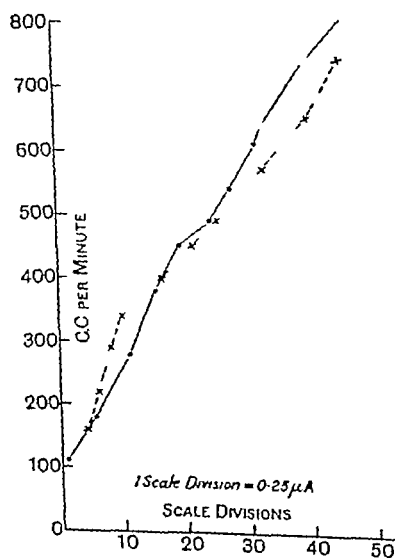
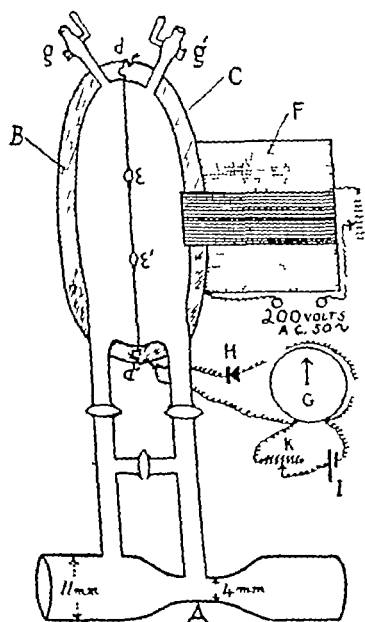
OF THE

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March 20, 1926

A blood velocity recorder By I DE BURGH DALY

The side tubes from a Venturi meter or from a Pitot tube (*A*) are connected to a specially constructed differential manometer consisting of two ebonite cups (*B*, *C*), the edges of which fit accurately against one another (*d*, *d'*). The surfaces of two thin sheets of rubber are stuck together with rubber solution and a coil of wire (*e*, *e'*) is enclosed between



them, this double membrane is clamped between the edges of the cups so that two separate chambers are formed. The edges of the cups fit together in such a way that when they are fixed together the double membrane is slightly and evenly stretched. The ends of the coil (*e*, *e'*) are brought to the outside and are not damaged by the clamping

Stop-cocks (g, g') are fitted to facilitate the filling of the apparatus. The iron core of a solenoid (F) projects a short distance into the interior of one of the cups. Variations of blood velocity in the Venturi meter or Pitot tube cause a difference in the pressures on each side of the double membrane thereby altering the distance between the coil (e, e') and the solenoid. An alternating current applied to the solenoid will induce a current in the coil (e, e') which may be rectified by a "firm contact" crystal (H) and then measured on a suitable galvanometer (G). When the pressures in each chamber of the manometer are equal to one another, the galvanometer shows a deflection which is brought back to zero by means of a compensating battery (I) and a resistance (K). It is convenient to connect the side tubes of the Venturi meter or Pitot tube so that an increase in blood velocity causes an increase in the current through the galvanometer. Variations in blood velocity of 20 c c per minute may be detected when a sensitive galvanometer is employed. Tests made by connecting the Venturi meter to a water tank, and measuring the outflow and the deflection of the galvanometer, show that the instrument, when calibrated, may be used for the quantitative estimation of blood flow. The solenoid F was not specially made for the apparatus and consisted of one pound of 22 s w g, D C C on an iron core 9 cm long and 1 cm in diameter. Coil e, e' was made up of 50 turns of 40 s w g (enamelled) wound on a 15 mm former. The current through the solenoid was 0.75 amp. Two calibration curves are shown in the graph, they were taken at an interval of five days and suggest that changes in the rubber membrane have taken place during this time.

The reaction between globin and hæmatin

By R. HILL and H. F. HOLDEN

Globin has been prepared, soluble over the range p_H 5-9 and free from hæmatin and denatured globin.

It reacts at any point within this range with added hæmatin to form methæmoglobin.

The oxyhæmoglobin thence obtained is spectroscopically indistinguishable from the original oxyhæmoglobin. Satisfactory spectroscopic observations necessitate the absence of denatured globin. There is no evidence that this condition has previously been realised.

This may explain the statements of earlier workers that a solution of hæmochromogen, free from hæmoglobin, in dilute alkali, yields some hæmoglobin on neutralisation.

Calcification in rabbits By MAY MELLANBY
and ESTHER M KILLICK

Since E Mellanby published in 1918 (*Phys Proceedings*, Jan 1918) a preliminary account of his experimental work on dogs, in which he showed that a fat-soluble vitamin played a dominant part in the ætiology of rickets, the subject has been extensively studied in rats. About two years ago, in the course of an investigation by one of us (M M) into the problem of the development of dental tissues and the incidence of caries, an opportunity was afforded of studying the problems in rabbits. The herbivorous habits of these animals made it possible to test also the calcifying properties of some green foods and root vegetables.

In attempting to find a suitable basal diet the results were at first complicated by several cases of scurvy. Lemon juice was chosen instead of white turnip as the standard antiscorbutic since it was impossible to get white turnip continuously. Animals fed on a daily ration of 4 parts of oats to 1 part of bran + 6 c.c. of lemon juice showed some signs of rickets. Growth was, however, poor. When 1.5 p.c. CaCO_3 was added to this diet, growth was much better, life was prolonged and bad rickets and defectively formed teeth usually resulted (16 cases out of 19). This would seem to be contrary to what might have been expected on the basis of the calcium-phosphorus ratio of the diet, the importance of which in the ætiology of rickets has been so strongly emphasised by some workers. The addition of the CaCO_3 brought this ratio nearer to the optimum said by some to prevent rickets in rats.

The improved growth brought about by the addition of CaCO_3 to the oats, bran and lemon juice seemed to be largely responsible for the greater intensity of the rickets. The evidence in favour of this is that, when one rabbit had x gram of this mixture daily and another of the same litter had $2x$ gram, the second grew well and developed rickets, while the first grew very little and was practically normal. It is therefore important in comparative experiments to keep the rate of growth of the animals the same.

Rabbits fed on oats, bran, CaCO_3 and lemon juice, without any other ingredient, lived up to seven months but did not develop caries although they had bad rickets. Various additions to the diet were therefore made with the object of prolonging the life of the animals to see if caries developed under the experimental conditions. The calcifying properties of certain vegetables were also tested.

Further results of the work were as follows

(1) Grass added to oats, bran and CaCO_3 , if given in sufficient quantity in spring or summer, tended to prevent the development of defective bones and teeth in late summer and winter the addition of grass resulted in a peculiar osteoporotic condition. Winter grass, even in fairly large quantities, sometimes allowed both rickets and scurvy to develop

(2) The addition of green cabbage to oats, bran and CaCO_3 , with or without lemon juice, seemed to improve the general health of the animals and allowed them to live longer but did not prevent severe rickets and defectively calcified teeth from developing. If the cabbage was boiled, rickets developed earlier and more severely even when the rate of growth was constant. Radiated cabbage prevented or delayed the onset of rickets

(3) White cabbage, white turnip and potato did not appear to assist calcification processes in any way

(4) Dandelion leaves, carrot and swede turnip, although giving somewhat varying results, all had more calcifying action than cabbage

(5) Both cod liver oil and egg yolk prevented defective calcification. After about two months of diets in which these substances were included the rabbits lost weight. This might be due to the intolerance of rabbits for fats. We are now using the unsaponifiable fraction of cod liver oil as the source of calcifying vitamin

(6) Exposure of rabbits on the basal diet to a mercury vapour lamp brought about great improvement in calcification

The presence in foodstuffs of substances having specific harmful effects under certain conditions By EDWARD MELLANBY

(1) A substance interfering with calcification

Interference with calcification of bones has been demonstrated to be a special property of cereals⁽¹⁾. When puppies eat diets deficient in the calcifying vitamin, the calcification of the bones becomes worse, the greater the amount of cereal eaten. There are also great differences among cereals themselves in their power to inhibit calcification⁽²⁾. Oatmeal, although containing more calcium and phosphorus than other cereals tested, is the most potent, and white flour the least potent, in this respect. Up to the present it has not been possible either to isolate or to gain any insight into the nature of the substance responsible for this effect. Some of its properties have been dealt with elsewhere. New

properties which seem to establish it as a definite entity are (1) if oatmeal is boiled with 1 p c hydrochloric acid until the starch is hydrolysed and the mixture is then neutralised with soda, the substance interfering with calcification is apparently destroyed (2) if oats are allowed to germinate for 1, 3 or 6 days (in each case after 2 days' steeping) and then heated at 100° C for 18 hours, the power of the cereal to prevent bone calcification is reduced Both germination and heat are necessary to get the best result, and the longer the time of germination the less does the product interfere with calcium deposition For instance, 6 days' germination and drying by heat results in a cereal product which is at least as good as and probably better than white flour as regards bone formation Heat alone at 100° C has practically no destructive effect and germination alone has but slight influence The same facts apply to barley and probably other cereals Germinated and kilned barley is comparatively good as a cereal product from the point of view of bone formation and much better than the original barley These results suggest most strongly that a substance in cereals having a positive interfering effect on calcification of bone has been destroyed Oats heated with 1 p c sodium hydroxide for one hour still contain the substance

(2) *A substance interfering with the nervous system*

A second type of specific harmful effect produced by diet is that in which severe nervous symptoms are produced In 1916 Hart, Miller and MacCollum⁽³⁾ described symptoms, not unlike those recorded for beri-beri, produced in pigs by diets containing wheat and wheat embryo They said these were due to a toxic substance in the fat of wheat embryo

If wheat germ is added to a diet deficient in fat-soluble vitamins to the extent of 10 p c of the cereal puppies often develop severe nervous symptoms after two or three months of the diet The symptoms may be so widespread that it is difficult to describe them. The most obvious is incoordination

The action is prevented by butter and cod liver oil and probably other sources of fat-soluble vitamin It is also reduced in intensity by the addition of calcium carbonate to the diet Boiling wheat germ for 1 hour in 1 p c hydrochloric acid also reduces the symptoms

In my experience the major nervous lesions are more central than those indicated by Hart, Miller and MacCollum, who described changes only in the anterior lower cells and seemed to think that they were dealing with a type of beri-beri

A condition of sub-acute combined degeneration of the spinal cord

may be produced in these animals and can be seen by Marchis' stain. The brains have not been examined yet for degenerative changes but the intensity of the nervous symptoms suggests that these will be large. The changes in the cord and the nervous symptoms are similar in many respects to those that develop in pellagra and ergotism and the experimental results suggest that these pathological conditions are due in part to a similar toxic factor eaten under conditions which allow the specific nervous effect to be produced.

The above are two instances in which harmful effects are apparently produced by dietetic ingredients under certain conditions. The effects of these substances are counteracted by fat-soluble vitamins and until their chemical composition is known might therefore be described as "Toxamins".

(1) E Mellanby Special Report Series, Medical Research Council Nos 61 and 93

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On the colorimetric determination of hydrogen-ion concentration By J H SHAXBY (Cardiff) and OLWEN M JONES

1 In measuring the hydrogen-ion concentrations of solutions of sodium bicarbonate and of blood serum Dale and Evans(1) found discrepancies between the values obtained by electrometric and colorimetric methods. The latter gave higher p_H 's, the difference being about 0.2. They attributed these differences to catalytic action at the hydrogen electrode, other workers(2) have put forward other explanations, but a further possible source of error appears to lie in the method of colorimetric estimation by the matching of the tints of two columns of liquid of unequal depths.

In the Dale and Evans comparator 1 c.c. of the solution to be measured is placed in one tube of the instrument, with about 4 drops of Neutral Red. In the adjacent similar tube is put 1 c.c. of a standard phosphate solution, with the same amount of indicator. A sufficient quantity of a second standard phosphate solution to give a match is then added to this tube, and the p_H calculated using Sorensen's data, from the relative amounts of the two standards. The final depth of solution in the second tube was from 1 to $2\frac{1}{2}$ times that in the first.

This method assumes the rigid applicability of Beer's Law, i.e. that the colour of a solution of given p_H is independent of the depth of solution used, so long as the amount of indicator remains constant and

is not so great as to influence the p_H . The increased depth is supposed to counterbalance the increased dilution.

2 It appeared desirable to test whether this is actually true in the conditions of these experiments. 1 c.c. of a standard phosphate solution of p_H 6.5 was placed in one cell of the comparator with 4 drops of Neutral Red, and $1\frac{1}{2}$ c.c. of the same in the other cell, likewise with 4 drops of Neutral Red. Similar tests were made with solutions of other p_H and of varied depths. In all cases the second cell showed the deeper pink colour, i.e. a greater absorption for the shorter wave-lengths. For the extra depth corresponding to the extra $\frac{1}{2}$ c.c. the difference in tint was considerable, and increased with increase of difference of depth up to about 3 c.c. with the solutions used. Obtaining matches by addition of a second standard phosphate solution to the second cell, the apparent differences of p_H proved to be about 0.1 or 0.2 using solutions of p_H 6.5 for the first filling, and of about 0.2 with solutions of 7.5.

Spectroscopic examination of the "matched" liquids showed in some cases that the apparent equality of tint was not exact, suggesting that the variation of tint resulting from the unequal dilution and the failure of Beer's Law is not precisely compensated by the p_H variation due to the addition of a second standard phosphate.

While the factor discussed above may not be the whole cause of the discrepancies found by Dale and Evans, it is one which must be guarded against in all colorimetric work in which columns of unequal depth are matched against one another. The error can be avoided in the case considered by adding more of the liquid under test to the first cell so that the depths in the two are approximately equal when a match is obtained. The only methods which are completely free from objection in this respect are those in which the comparison chambers are of equal depths, as pointed out on general grounds by Stanford (3).

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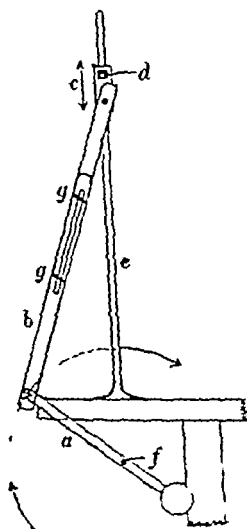
Concerning the part played by the sinus caroticus in the central regulation of the circulation By R A NASH (*From the Institute of Physiology and Biochemistry, University College, London*)

By means of the innervated heart-lung preparation it can be placed beyond doubt that a rise of blood-pressure in the head causes a slowing of the heart rate and a fall of blood-pressure in the body. In a recent preliminary communication H E Hering has suggested that these effects are not due to a direct effect of blood-pressure upon the centres, but to a reflex originating within the sinus caroticus, the afferent impulses being conducted along a small branch of the glossopharyngeal nerve. Hering claims to have shown that all effects of change of blood-pressure in the head disappear after section of the sinus nerve, or after exclusion of the sinus from the circulation. It was thought interesting to test these conclusions with the use of the innervated heart-lung preparation. Experiments were made in which the perfusion of the brain was carried out either through the vertebral arteries, with complete exclusion of the sinus caroticus on both sides, or through the internal carotid arteries well beyond the sinuses. In both cases the fact of exclusion of the sinuses from the circulation was verified by subsequent dissection, and in both cases it was found that changes in the cerebral pressure exerted their usual definite effects upon heart rate. By means of cerebral perfusion in the whole animal with exclusion of both sinuses and with cut vagi it was found that a rise in the cerebral blood-pressure still determined a fall of blood-pressure in the body. With regard to Hering's statement that pressure upon the sinus caroticus produces a similar effect to rise in blood-pressure, his results can be entirely corroborated. However, the effects of changes of blood-pressure in the head must be considered to depend not only upon a reflex from the sinus caroticus but also upon another more central effect.

PROCEEDINGS
OF THE
PHYSIOLOGICAL SOCIETY,
May 15, 1926

A labour-saving device for use in gas analysis
By F A DUFFIELD

The absorption of oxygen by pyrogallate solution in gas analyses is a lengthy process and in actual practice it is always a tedious undertaking. Its common use in the investigation of physiological processes is a reason for giving details of a procedure whereby this disadvantage



may be eliminated and for considering the method one of general interest. A mechanical means of promoting the absorption of oxygen has been devised and since then it has been in constant use in the Physiological Laboratory in the University of Liverpool.

The apparatus consists of a metal crank *a*, 8 ins in diameter, and this is attached by a bolt to a moving adjustable arm *b*, the greatest length to which this is capable of extension is 35 ins The upper end of the arm is similarly connected to a sliding block *c* and this is bored to admit the steel rod *e* The block *c* moves vertically up and down on the rod, the latter being kept well oiled The crank is driven from its axle *f* through a series of pulleys by an electric motor not shown in the diagram When the motor is started the crank *a* rotates in the direction indicated by the arrows and consequently the block *c* moves up and down the actual extent of its movement is fixed by the length of the adjustable arm and this length may be increased or diminished after loosening the thumb-screws *g* The levelling tube of the Haldane apparatus is held by a clamp *d* firmly fixed to the block *c* The rate of the vertical movement of the levelling tube can be varied by the inclusion of an adjustable resistance in the wiring circuit of the motor The apparatus has been made by Mr C R Nisbett of this Department and in one instrument which he made bicycle chains and cog-wheels have been substituted for the belts and pulleys a device which does away with the necessity of giving any attention to the belts, though it creates some noise when running

Dr Haldane states in his *Methods of Gas Analysis* that when the air has been driven over into the pyrogallate pipette once or twice nearly all of the oxygen will have been absorbed My own experience is that about a quarter of an hour is needed for its absolutely complete absorption In a series of analyses the continuous handling of the levelling tube is very tiring and when a number of these are to be undertaken in one day there may be a tendency to shorten the process at the expense of the accuracy of the results It is claimed therefore that the employment of a mechanical means of promoting the absorption of oxygen not only reduces the fatigue incurred by an exceedingly monotonous procedure, but in an indirect way lessens the errors of gas analysis

Factors influencing the foetal respiratory centre

By A ST G HUGGETT

The animals experimented on were the foetuses of goats The mother, 14-30 days before full-term, was anaesthetised by urethane and ether and immersed in a bath of saline at 38° C where Caesarean section was performed The foetuses were removed from each horn of the uterus separately, the placenta being left intact The foetus was handled as little as possible and kept immersed in warm saline with its snout always

below water. In this way the stimulus to respiration was minimal and no gases were exchanged *via* the respiratory tract. The result was a condition approximating to that *in utero*, but allowing of experimental work on the animal.

Clean skin incisions with a scalpel produce no respiratory movements, foetal apnoea being constant. No evidence was found at any time to support the view of Ahlfeld, that feeble respiratory movements take place *in utero* normally.

Cutting of one vagus produced no effect on respiration or heart-beat. Stimulation of the central or cut vagus with a tetanising current produced no effect, but stimulation of the peripheral end caused slowing of the heart after a latent period of 2 seconds.

Cutting right sciatic nerve caused no respiratory movements but did cause muscular movements both reflex and direct. Stimulation of the central end caused reflex muscular movements and also initiated slow respiratory movements of the thorax, lasting as long as the nerve stimulation lasted, but stopping with it.

Stimulation of the central end of the vagus while these respirations due to sciatic stimulation were occurring, caused these inspirations to become slow. They quickened again on removal of the vagal stimulus, the sciatic stimulus continuing the whole time.

It seems, therefore, that the foetal respiratory centre has a normal but sluggish response to nerve stimuli which affect the adult centre. That is to say, it has a high threshold value for nerve stimuli. This lack of response to stimuli is not due to cerebral inhibitory impulses, because decerebration has no effect on foetal apnoea, and after decerebration the respiratory centre still responds to the above stimuli.

Clamping the umbilical cord initiated slow respirations which ceased with removal of the clamp.

Injection of 1 cc of 1 p.c. lactic acid intravenously had no action on the quiescent respiratory centre but caused a slowing of the heart.

Injection of 1 cc of 5 p.c. ethyl acetate caused respiratory movements which were very slow, occurring at intervals of 15, 20, 20, 40, 51, 62 and 75 seconds till foetal apnoea supervened again.

Strong chemical stimuli will therefore produce a similar result to that occurring in the adult when the stimulus lasts.

It seems, therefore, that the foetal centre has a definite inertia preventing response to normal stimuli unless they are very strong. This inertia may be a developmental or it may be due to some inhibition of the centre.

PROCEEDINGS
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**Experiments on the degeneration of striated muscle fibres
after sympathetic denervation** By W FELDBERG, *Berlin*

In the course of some experiments on the ear-vessels it was necessary to remove the superior cervical ganglion in several rabbits and to wait some time for degeneration of the sympathetic nerve fibres. The late Prof Langley therefore suggested that I should examine simultaneously some of the ear-muscles to see if I could find histologically the signs of degeneration described by Hunter and Royle⁽¹⁾. Hunter⁽¹⁾ put forward the view that the individual muscle fibre was not doubly innervated, but that some of them have a sympathetic and others a somatic innervation. In support of this view he quoted the results of Kulchitzky, who found that (in the snake) the medullated and non-medullated fibres do not terminate in the same muscle fibres. Royle examined histologically the muscles of the hind limbs of the goat, on one side the lumbar sympathetic chain was removed previously, the other side was used for control. "The preparations from the operated side showed in transverse section a number of small muscle fibres which were in marked contrast to the fibres of average size in the same preparation, which were apparently normal. These diminutive fibres were too numerous to be accounted for as the conical or pointed terminations of normal muscle fibres. Further, the connective tissue (endomysium) between the individual muscle fibres has increased in amount." From these results he drew the conclusion that the diminutive fibres were muscle fibres normally supplied by the sympathetic.

We made experiments on 12 rabbits. A fortnight to three months previously the superior cervical ganglion had been removed and in two animals also the ganglion stellatum of the same side. We examined three muscles from the anterior surface and the side of the ear, these should be especially fit for studies on sympathetic innervation as they play a great rôle in maintaining the erection of the ears. The muscles

were the musculus fronto scularis, which is continued by the anterior scuto auricularis superior, the musculus temporo auricularis and maxillo auricularis. The fibres of the first of these muscles run in more oblique direction, so that it is difficult to get good transverse sections. Later on, therefore, we used especially the two other very small muscles. The same muscles of the normal side were used as control. They were fixed in 10 p.c. formalin, and transverse sections in the middle of them were stained in hæmatoxylin and van Gieson.

We could not confirm the results of Hunter and Royle obtained on the goat. We could not find any difference in the size of parts of the muscle fibres between the normal and the operated side. In two preparations it seemed as if the connective tissue between the individual muscle fibres had increased on the operated side, but we could not confirm this increase on the following ten experiments. In some experiments we made a drawing with the camera lucida and counted the smaller muscle fibres but did not obtain different results on each side.

According to Royle's description the diminutive muscle fibres lie together in groups. We found that in the musculus temporo auricularis and especially in the maxillo auricularis the fibres in the periphery of the operated as well as of the normal side were mostly the largest, while the fibres in the middle of the muscle were considerably smaller in diameter. But there was no distinct difference in both sides.

At the end of our experiments the experimental and critical paper of Garven(2) appeared. This author could not confirm the results of Kulchitzky. He found in the panniculus carnosus of the hedgehog that all the muscle fibres appear to be innervated by medullated nerves, and that in some cases a medullated and non-medullated (accessory) nerve fibre ended in the same muscle fibre. Our results show at least that the observations of Hunter and Royle cannot be regarded as true for all muscles without further experimental evidence.

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The sources of energy in ontogenesis By JOSEPH NEEDHAM

There is much evidence in the literature of embryonic metabolism which goes to show that during its development, the embryo makes use of foodstuffs as energy-sources in the order carbohydrates, protein, fat. In order to develop this conception, experiments have been made on the

chick embryo in which various substances have been estimated during its incubation period

It has been found that there is a period of intensive urea production from the fifth to the ninth day. After that point the excretion of urea no longer keeps pace with the growth and differentiation of the embryo. There is an exactly similar period of intensive uric acid production between the seventh and the eleventh days, so that the point of maximum intensity of uric acid production occurs two days later than the point of maximum intensity in the production of urea. The adult hen excretes about 95 p.c. of its nitrogenous waste in the form of uric acid, and this condition is reached in the developing embryo about the ninth day. Before then urea greatly predominates in the excretion of nitrogen.

From the figures for uric acid and urea, the protein combusted each day was calculated. The point of maximum intensity in the combustion of protein is attained at 8.5 days of development, and this is very significant because the combustion of carbohydrate is associated with the first five days, and that of fat with the last ten. Comparison with the data obtained by earlier workers leads to the conclusion that the protein used as a source of energy belongs entirely to the coagulable fraction. The total protein nitrogen lost during development by combustion amounts to 7.5 p.c. of the total protein nitrogen present at the beginning, and to 3 p.c. of the total foodstuff burnt. These figures compare in a very interesting way with others obtained for other species of animals by earlier observers. It has also been possible to calculate from the results of purely chemical analyses the respiratory quotient, and this agrees as well as can be expected at present with the experimentally determined respiratory quotients of Bohr and Hasselbalch and of Lussanna.

Injection experiments and other considerations lead to the conclusion that factors located in the embryo decide what the embryo shall make use of as a source of energy. It does not, for example, combust fat because its protein supply has been exhausted.

The polariscopic appearance of colourless "crystals" of hæmoglobin By D FRASER HARRIS

Through the kindness of Sir William Bragg and his assistant Mr W T Astbury of the Davy-Faraday Laboratory, I am enabled to report on the appearance of the colourless "crystals" of hæmoglobin of the guinea-pig described at the meeting on February 20th, 1926. Viewed in the field of the "crossed nicols," they appear uniformly dark as the nicols are rotated.

Such of the coloured tetrahedra as have remained sufficiently transparent to transmit some light, appear distinctly although not brilliantly doubly refractive or birefringent

The true tetrahedra of the "cubic" class of crystals are normally singly refractive, but such crystals if strained may develop the property of double refraction

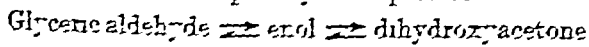
If the precursors of the white forms were at any time birefringent they have now lost this property. These white angular masses are most probably "pseudo-morphs," that is they are masses of powder either microcrystalline or truly amorphous, representing the protein basis of the original hæmoglobin crystal, which have retained the external form and angles of the tetrahedron. There is just a possibility that the white tetrahedra are still single crystals just as the original, but are now isotropic (cubic) instead of birefringent—although this is not at all probable

The effect of glyceric aldehyde and dihydroxyacetone on insulin hypoglycæmia By H. G. REEVES and J. A. HEWITT

The mechanism involved in the degradation of glucose, in the animal, with the ultimate production of carbon dioxide and water, still remains open to considerable discussion. That lactic acid is an intermediate product in this biological oxidation is now fairly certain, but what the precursor of lactic acid is awaits convincing proof

Accumulated evidence justifies the assumption that at least two substances, viz. glycine aldehyde and dihydroxyacetone, may intervene between glucose and lactic acid.

Dakin⁽¹⁾ has stressed the possibility of the existence of a labile intermediate and the present authors⁽²⁾ have proved that mild alkalinity causes glycine aldehyde and also dihydroxyacetone to give rise to an unstable enol. The relationship may be expressed



This reaction is analogous to the familiar interconversion of glucose and fructose in the Lohry de Bruyn transformation, and furthermore Schmitz⁽³⁾ has prepared from glycine aldehyde, α -ketone, an inactive form of fructose

On these considerations it seemed probable that glycine aldehyde and dihydroxyacetone would be interchangeable in the body and that some information could be obtained as to the efficiency of these two substances in raising blood sugar, glycogen both in mice and rabbits

The animals were rendered hypoglycæmic and the typical symptoms developed. Then by subcutaneous injection the efficiency or otherwise of the glyceric aldehyde or the dihydroxyacetone was ascertained. In each case control experiments were conducted and where necessary glucose was administered.

Glyceric aldehyde displayed *no* beneficial action in either mice or rabbits even when given in large doses. With mice, very exceptionally, a slight transitory recovery was observed but relapse always ensued and recourse had to be made to glucose. This transitory improvement was not noted in rabbits. On the other hand, dihydroxyacetone on similar animals immediately allayed the hypoglycæmic symptoms and was in every way as efficient as glucose. Thus in mice and rabbits hypoglycæmia is relieved by dihydroxyacetone but not by glyceric aldehyde and it is clear that the hypoglycæmic rodent cannot effect the conversion of the aldehyde into its isomeric ketone.

Apart from remarking that dihydroxyacetone is a possible intermediate in carbohydrate metabolism it is difficult to offer a satisfactory explanation of the above results. However, the following suggestions seem not unreasonable, viz. that whether the convulsions be relieved by the glucose itself or by some derivative thereof, then dihydroxyacetone is capable, in the body, of being converted into this substance and that glyceric aldehyde cannot be thus transformed.

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The action of light on cod-liver oil

By P. R. PEACOCK and S. WRIGHT

Normal cod-liver oil fluoresces brightly in a beam of ultra-violet rays from which the visible spectrum has been excluded by means of Wood's glass.

This fluorescence is, as usual, associated with marked absorption of the ultra-violet spectrum.

If such oil is exposed to bright light, the fluorescence progressively disappears.

The length of exposure required depends on the intensity of the light source and on the thickness of the layer of oil employed.

This effect has been termed "delumination" and corresponds with proportionate increase in the transmission spectrum of the oil

Whereas normal cod-liver oil absorbs the whole ultra-violet spectrum, the deluminated oil transmits up to the limit of the sun's spectrum (3100 Å ν)

Normal cod-liver oil, as shown by Rosenheim and Drummond, gives an ultramarine colour with arsenic chloride, which has been correlated by these workers with the presence of vitamin A

Oil which has previously given this reaction, after irradiation, fails to do so

It appears, therefore, that the vitamin A in cod-liver oil is destroyed by exposure to light

Animal experiments are in progress with the object of confirming this view

Experimental evidence suggests that there is more than one fluorescent body present in cod-liver oil, and that vitamin A may be identical with one of these

Full technical details will be given in another publication

ADDENDUM

A preliminary series of feeding experiments on young rats gave the following results. The control animals fed on the usual basal ration plus 2% normal cod liver oil increased in average weight from 43 g to 118 g at the end of 60 days, or a percentage increase of 175%. Animals fed on the same basal diet to which 2% deluminated oil had been added grew more slowly and had increased in average weight in 45 days from 42 g to 92 g. The weight then began to fall gradually and on the sixtieth day was only 85 g. Definite xerophthalmia was then shown by all these animals and their general condition was poor. Normal cod liver oil was substituted for the deluminated material. The eye condition was greatly improved at the end of 4 days and the animals increased in weight to 98 g. The feeding experiments thus confirm the finding that vitamin A in cod liver oil is destroyed by sunlight

The isolation of secretin By J MELLANBY

(Preliminary communication)

The method is based on three facts (1) secretin is present in a pre-formed condition in the mucous membrane of the small intestine, (2) secretin may be extracted from the mucous membrane by absolute alcohol at room temperature, (3) secretin is absorbed from solution by bile salts

The mucous membrane from the duodenum is ground up with four volumes of absolute alcohol and filtered. The alcohol is distilled from the filtrate (*in vacuo*) until it becomes opalescent. The residual fluid is now diluted with two volumes of water and slightly acidified. A variable but often fairly heavy precipitate is produced. Flocculation of this precipitate is assisted by the addition of magnesium sulphate to the extent of 1 p.c. to the solution. This precipitate contains only a trace of secretin and may be removed by filtration or centrifugalisation. A solution of bile salt or sodium cholate is added to the extent of 2 p.c. to the clear fluid. The bile salt, being precipitated in acid solution, absorbs the whole of the secretin. The precipitate consisting mainly of bile salt and secretin is obtained as a compact mass by spinning in a centrifuge and washed with acetone and ether. The product is purified by dissolving in a minimal quantity of 80 p.c. alcohol and reprecipitating the secretin from this solution by the addition of an equal volume of acetone. The activity of the final product is such that 0.3 mgm. injected intravenously into a cat caused the secretion of 3 c.c. of pancreatic juice. Secretin is probably a polypeptide. It is readily soluble in water and this solution gives a faint biuret reaction and a well-marked Pauly reaction. Since Millon's test is negative this probably indicates that secretin contains histidine or some derivative of it. It is very rapidly destroyed by pepsin and trypsin. Heating to 100° C. with NaOH (1 N) for five minutes destroys it, similar heating with HCl (1 N) destroys 50 p.c. of it. Pure secretin has no effect on the blood sugar of a rabbit. Therefore its action is limited to the external secretion of the pancreas. It has no depressant action on arterial blood pressure.

